ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH

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NATIONAL INSTITUTE OF ALLERGY
AND INFECTIOUS DISESASES

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ANNUAL REPORT OF PROGRAM ACTIVITIES

U.S. National Institute of Allergy and Infectious Diseases

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INDEX

ANNUAL REPORT

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Director, NIAID 1

Extramural Programs 1

Intramural Programs 1

Laboratory of Clinical Investigation

Summary 1

10 (c) - Office of the Chief 8
11 (c) - Mechanisms of Fever 9
12 (c) - Respiratory Viral Diseases 14
13 (c) - Antimicrobial Drug Therapy 18
14 (c) - Staphylococcal Diseases 23
15 (c) - Hepatitis and Mononucleosis 24
16 (c) - Aseptic Meningitis 25
17 (c) - Allergy-Immunology 26
18 (c) - Enteric Diseases 31
19 (c) - Clinical Investigations on Helminthic Diseases 33
20 (c) - Clinical Investigations in Protozoan Infections and Diseases 34
21 (c) - Cystic Fibrosis of the Pancreas 44
22 (c) - Basic Biochemical Studies 45
23 (c) - Systemic Fungal Diseases 49
24 (c) - Sarcoidosis 53
25 (c) - Pyelonephritis 55
26 (c) - Urinary Tract Viruses 56
27 (c) - Biochemical Studies on Staphylococcal Cell Walls 59
28 (c) - Pathogenesis of Viral Infections 60
29 (c) - Mid-Line Granuloma and Wegener's Granulomatosis 63
30 (c) - Studies on Immediate and Delayed Sensitivity to Simple Chemicals and Protein 65

Laboratory of Infectious Diseases

Summary 1

60 - Studies on Cellular and Cell-Free Staphylococcal Penicillinase 37
60-A - Antibiotic Activity of Sea Water 40

61 - The Electron Transport System in an Autotrophic Hydrogenomonad 43
62 - Biochemical Studies on Staphylococcal Cell Walls 46
63 - Non-specific Immunity Associated with Serum Siderophilin and the Iron Metabolism of both Pathogen and Host 50
63-A - Biochemistry of the Acquisition of Iron by Mammalian Tissues as Mediated by the Iron-binding Protein, Siderophilin 54
63-B - Coliform and Pseudomonas Urinary Tract Infections Refractory to Antibiotic Drug Therapy 57
64 - Detoxification of Potential Tuberculostatic, Fungistatic, Parasiticidal and Viricidal Agents 58
64-A - Potential Fungistatic, Parasiticidal, Leprocidal and Viricidal Agents 62
64-B - Isolation, Purification and Characterization of Antibacterial and Antiviral Substances from Mollusks 64
65 - The Biology of Group A Streptococci 67
66-A-J - Epidemiologic Studies of Illnesses and Microbial Experience of Junior Village Nursery Children 71
66-K - Epidemiological Studies on Efficacy and Safety of Influenza Vaccine in General Population Groups 80
66-L - Epidemiological Studies on Efficacy and Safety of Asian Influenza Vaccine in General Population Groups 1957-1958 82
66-M - Hemagglutination Inhibition (HI) Antibody to Type A and B Influenza 8-9 years after Vaccination with 100 CCA Units of Monovalent Mineral Oil Adjuvant Vaccine 85
66-N - Inoculation of Parainfluenza Virus Type 3 in Adult Volunteers 88
66-O - The Etiology of Respiratory Disease on a College Campus 91
66-P - The Role of Viruses in the Etiology of Ear Disease ... 93
67 - Studies on Enteroviruses, Adenoviruses, Reoviruses ... 94
67-A - Studies on Viruses of the Enteric Tract of Cattle 99
67-B - Studies on Eosinophilic Meningitis in the Pacific Area 102
67-C - A Study of Squamous Cell Carcinoma (Cancer Eye) in Cattle 106
68 - Studies of Tumor Viruses in Nature 108
68-A - Sero-epidemiology of Virus Infections 114
69-A - Study of non-bacterial Respiratory Disease A) Children 117
69-B - Study of Non-Bacterial Respiratory Disease B) Adults: Military Populations 122
70-A - Laboratory Studies of Newly Recognized Viruses Associated with Respiratory Illness A) Eaton Agent, Para Influenza Viruses, and Respiratory Syncytial Virus 126

70-B - Laboratory Studies of Newly Recognized Viruses Associated with Respiratory Illness B) Enteroviruses 130
71 - Studies of Tumor-producing Viruses 133
71-B - Studies of Mouse Viruses with Special Reference to Their Importance as Extraneous Viruses ("Background Nois") in Mouse Tumor Systems 136
72-A - Study of Respiratory Viruses in Human Volunteers A) Enteroviruses and Respiratory Syncytial 141
72-B - Study of Respiratory Viruses in Human Volunteers B) Eaton Agent and Adenoviruses 145
76-A - Ecologic Studies of Fungi Pathogenic to Man 148
76-B - In vivo Tests of Antimycotic Drugs and Antibiotics ... 151
76-C - Identification and Study of New and Unusual Fungi from Mycoses 154
78-A - Biochemistry and Physiology of Pathogenic Fungi. (In vitro Studies of the Action of Antifungal Agent on Pathogenic Fungi) 157
78-B - Studies on the Physiology of Coccidioides Immitis 160
79-A - Immunity Studies with Pathogenic Fungi 162
79-B - Effect of Physiological Alteration on the Pathogenesis of Yeast Infections 165
79-C - Antigenic Studies on Candida 168
79-D - Studies on Cryptococcus 171
79-E - Studies on the Toxicity of Canadida albincans 173
79-F - Role of the Reticulo-Endothelial System in Immunity Against Pathogenic Fungi 175

Laboratory of Biology of Viruses

Summary 1

80 - Mechanism of Oncogenic Activity of Tumor Viruses 5
80-A - Rabies Prophylaxis 8
81 - Genetics of Animal Viruses and Cells 11
82 - Mechanisms of Recovery from Viral Infection 13
83 - Genetics of Animal Viruses 16
84 - The Metabolism of Normal and Virus Infected Animal Cell Cultures 19
84-A - Effects of Fluorouracil Incorporation into Poliovirus 22
85 - Replication of Viruses 24
85-A - Relationship of Ribosomes to Viral Synthesis 28
86 - Biology of Mitochondria and its Relation to Endogenous and Viral Diseases 31
86-A - Characterization of Mitochondria 35
86-B - Investigations of Interference with Enzymatic Functioning of Mitochondria as a Mechanism for Carcinogenesis 39
86-C - Demonstration of Glucose Metabolism and Peptide Bond Formation by Isolated Brain and Liver Mitochondria 41

86-D - Comparison of the Properties of Crude and Crystallized Coxsackie A-10 Virus, of Cloudman S_{91}, Mouse Melanoma and of Mouse Muscle Nucleoprotein 43
87 - The Mechanism of Production of Poliovirus in Cultured Mammalian Cells 45
88 - Mechanism of Drug Resistance in Cultured Mammalian Cells 48
89 - Kinetics and Sites of Coxsackie Virus Multiplication 51
89-A - Virus Structure 54
89-B - Methods for Study of Nucleic Acid Structure 56

Laboratory of Tropical Virology
Middle America Research Unit
Arthropod-Borne Virus Section

Summary 1

93 - Studies on the Epidemiology of Arthropod-Borne Virus Infections 5
94 - Study of Arthropod-Borne Viruses in Tissue Culture ... 8
95 - Development of Experimental Procedures to Improve Serological Detection of Arthropod-Borne Virus Infections 10
96 - Grouping of Arthropod-Borne Viruses with Polyvalent Antiserum Pools 12
97 - Studies on Newly Recognized Groups of Arthropod-Borne Viruses. (1) The Bunyamwera Group 14
99 - Studies on Attraction and Feeding Behavior of Biting and Bloodsucking Arthropods in Relation to Preventive Measures 17
100-A - Epidemiological Investigations and Surveillance of Infectious Disease Situations in Panama and Central America 20
100-B - A Clinical and Virological Study of Oropharyngeal Lesions in Panamanian Children 26
100-C - Venezuelan Equine Encephalomyelitis in Panama 28
100-D - Serological Investigation of Measles Virus in Isolated Populations (in Panama) 32
100-E - Vesicular Stomatitis Virus (VSV) Infections in Panama 34
101-A - Virological Aspects of a Cooperative Investigation on the Ecology of Arthropod-Borne Viruses 37
101-B - An Appraisal of Chiggers and Other Acarina of the American Tropics for Pathogenic Agents 42
101-C - Viral "Jungle Fever" in U.S. Military Personnel 44
102-A - Intestinal Viral Flora in Rural Guatemalan Children A Survey of Three Villages 46
103-A - Use of Filter Paper Discs for Virus Isolation and Serological Testing 49

105-A - Studies of Microbial Diseases of Military Importance in Latin-America 53
108 - Studies of Histoplasmosis on the Isthmus of Panama 57
109 - Studies of Superficial and Deep Mycoses in Panama and Central America 63

Laboratory of Germfree Animal Research

Summary 1

110 - Pathogenesis of Amoebiasis 6
111 - Viral Studies in Germfree Animals 9
112 - Biology of Germfree Animals 11
113 - The Role of the Eosinophilic Leukocyte in the Inflammatory Response to Allergic Reactions and Parasitic Infestation 14
114 - Studies on Bacterial Interactions and Host-Bacteria Relations in the Germfree Animal 16
115 - Studies on the Pathogenesis of Infectious and Other Disease Processes in the Germfree Animal 20
116 - Studies on Helminthic Infections in Germfree Hosts 22
117 - Studies on Auto-Immune Diseases and Related Immunologic Processes 24
118 - Tumor Studies in Germfree Mice 28

Laboratory of Parasitic Diseases

Summary 1

120 - Administration and Research Planning and Coordination 8
121-A - Host Parasite Relations in Worm Infections in Laboratory Animals 9
121-C - The Biology and Control of the Snail Intermediate Hosts of the Schistosomes 13
121-D - Investigation of Intermediate Hosts and Vectors of Human Diseases Caused by Worms 16
122-A - The in Vitro Culture of Parasitic Helminths 19
122-B - The Development of Helminths in Germfree Animals 23
122-C - The Role of Helminths in the Etiology of Eosinophilic Meningitis and Meningo-encephalitis 25
122-D - Effects of Nutrition on Chemotherapy of Parasitic Diseases 28
122-E - Effects of Improving the Nutrition of Malnourished People Infected with Schistosoma Mansoni 31
122-F - Studies on the Influence of Dietary Factors on Experimental Parasitic Infections 35
122-G - Studies on Entamoeba Histolytica and Other Parasitic Protozoa 38

122-H - Biochemistry of Parasitic Protozoa 42
123-A - Fundamental Physiological and Biochemical Studies on Parasites, Intermediate Hosts, and Parasitized Animals 45
123-B - Pathological Physiology and Histochemistry of Parasitic Diseases 49
123-C - Biochemical Mechanisms of Energy Metabolism in Normal and Parasitized Animals 52
123-D - Biology of Trypansomes 56
124-A - Effect of Radiation on the Snail Hosts of Schistosoma Mansoni and on Schistosome Cercariae 59
124-B - Pathology of Acute and Chronic Schistosoma Mansoni Infection in the Mouse, Hamster, and Multimammate Rat (Mastomys) 61
124-C - Effect of Gelatin on the Rate of Clearance of Colloids from the Blood 64
125-A - Studies on Toxoplasmosis 66
125-B - Serology of Toxoplasmosis 71
125-C - Trichomoniasis 73
125-D - The Pathophysiology of Schistosoma Mansoni Infection in the Experimental Animal 76
125-E - Ammonia Metabolism and Toxicity in Relation to Liver Disease 79

Laboratory of Parasite Chemotherapy

Summary 1

130 - Administration, Research Planning and Coordination 5
131 - Experimental Chemotherapy of Schistosoma Mansoni 10
131-A - Chemotherapy of Plasmodium Gallinaceum and P. Berghei Infections 12
131-B - Effect of Nutritional Status of the Host on the Chemotherapy of Malaria 14
131-C - Protein Synthesis of Plasmodium Berghei in Vitro and in the in Vitro and in Vivo Binding of Chloroquine 17
132 - Studies in Human Malaria 20
132-A - Chemotherapy of Intestinal Parasites 24
132-B - Physiology of Parasites, with Special Emphasis on the Effect of Chemotherapeutic Agents 28
132-C - Virus-Parasite Associations 31
132-D - Development of Human Pathogens in Immature Insects .. 35
132-E - Insect Tissue Culture 38
133 - Studies of the Biology of Simian Malaria and of Human Malaria in Simians 40
133-A - Immunological Studies of Malaria Parasites 43
133-B - Tissue and Cell Cultures 46
133-C - Biochemical Studies of Simian Malarias 48
134 - Studies on Simian Malarias in Human Volunteers 50

134-A - Studies in Human Malaria 54
135 - Studies of the Vectors of Simian and Human Malarias . 58
135-A - Study of Cross Transmission of Simian Malaria to Man Under Field Conditions 62
135-B - Systematic Studies of the Malaria Parasites of Monkeys and Apes of Malaya and Nearby Territories 64

Laboratory of Immunology

Summary 1

140 - Studies in the Transplantation of Immunologically Competent Tissues 5
141 - Studies on Antibody Production and Mechanisms of Hypersensitivity 8
142 - The Immunology of C_3h Mouse High Line Sarcoma 11
145 - Studies of Auto-Antibodies in Human Disease States and Experimental Animals 13
145-A - Study of the Antigens in Serum by Immunochemical Techniques 18
147 - Immunochemical and Immunogenetic Studies of the Protein Allotypes in Serum 20
147-A - Immunochemical Studies on Human Serum Antigens and Antibodies 26
148 - Basic Studies on the Cellular Localization of Antigens, Antibodies and other Substances by the Fluorescent Antibody Technique and Fluorescence Microscopy 29
150 - The Relationship of Delayed and Immediate Hypersensitivity to Allergic Thyroiditis in Inbred Guinea Pigs. 34
151 - The Purification and Characterization of a Proteolytic Enzyme of Human Spleen 37
152 - The Chemistry of Antibodies 39
153 - Basic Studies on the Chemical, Physical and Skin Reactive Properties of Extracts of House Dust 43
154 - Immunocytochemical Studies of the Reactivity of Human Serum Proteins with Human Tissues in Normal and Disease States 46
155 - Cellular Production of Gamma Globulin; Studies of Rabbit Allotypes Using the Fluorescent Antibody Technique 48
156 - The Study of Delayed Hypersensitivity _in Vitro_ 50

Rocky Mountain Laboratory

Summary 1

170 - Office of the Director 8
171 - Rickettsial Infections 9
172 - Q Fever 14
173 - Selected Zoonoses of Regional Importance 21
174 - Transmission of Disease Agents by Certain Vectors ... 29

175 - Mechanisms of Hypersensitivity and Its Role in Disease 35
176 - Viruses, Virus Components, and Virus Surfaces 43
177 - Immune Prophylaxis of Mycobacterial Infections 49
178 - Immune Prophylaxis of Salmonella Infections 52
179 - Morphological Elements of Microorganisms in Immunity and Related Phenomena 56
180 - The Encephalitides 61
181 - Colorado Tick Fever 67
182 - Epidemiology of Human Infections of Special Interest 71
183 - Comparative Pathology 74

Laboratory of Bacterial Diseases

Summary 1

200 - Brucellosis 3
201 - Basic Studies on Staphylococcus 7
202 - Identification of Pseudomonas 9
204 - Intracellular Parasitism 12
205 - The Role of Infection in the Delayed Deaths of Mice Following Extensive Burn Injury 14
206 - Bacterial Food Poisoning 17

DIRECTOR - NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Summary 1

Annual Report of Program Activities

National Institute of Allergy and Infectious Diseases

January - December 1961

OFFICE OF THE DIRECTOR

1. Both the intramural and extramural activities of the National Institute of Allergy and Infectious Diseases have expanded during the calendar year 1961, as increased funds have become available.

2. A grant for the fifth International Center for Medical Research and Training was awarded to the Louisiana State University School of Medicine. The overseas affiliate is the Costa Rican School of Medicine, University of Costa Rica, and the Hospital San Juan de Dios.

3. Outstanding achievements in the intramural area of activity include the demonstration that a natural malaria infection of Malayan monkeys is transmissible to man under laboratory conditions. A field laboratory has been established at Kuala Lumpur to investigate the possibilities of transfer to man occurring in nature.

4. Our virologists, in collaboration with clinical investigators, have established in human volunteers that some fourteen different viruses are capable of producing the symptoms of upper respiratory disease, the "common cold." Using the same procedures, it was possible to show that the Eaton agent, known to cause primary atypical pneumonia, is really a sub-bacterial PPLO (pleuro-pneumonia-like-organism) cultivable on synthetic media. It is not a virus, as had been previously supposed.

5. To implement our interest in expanding virologic research, the decision, based upon discussions in 1961, has been made for this Institute to support the production, certification, and distribution of standard viral diagnostic reagents to qualified virologists.

6. Notwithstanding the probability that the number of etiologic agents for the common cold will be enlarged by future research, the decision was reached in 1961 to attempt the production of a polyvalent prophylactic agent or agents against upper respiratory disease. This will be based largely upon the recent researches of our own intramural virologists, and upon those of our grantees, amplified, it is hoped, by the cooperation of the pharmaceutical industry in producing prototype vaccines, and of other agencies experienced in field testing such biologicals.

7. One of the more important advances in the field of public and personal health during 1961, which was supported by this Institute, was the development of an effective measles vaccine by one of its long-time grantees, Dr. John F. Enders of the Children's Hospital in Boston, Massachusetts. This has been hailed almost universally as an achievement of outstanding merit.

8. As a part of the United States effort to establish better scientific communication with the USSR, this Institute sponsored the U.S. Infectious Disease and Microbiology Exchange Mission. A group of well known virologists, headed by Dr. Robert J. Huebner, Chief, Laboratory of Infectious Diseases, NIAID, visited research institutions and laboratories in Russia for four weeks in the spring. Members of the delegation were:

- Dr. Robert M. Chanock, Laboratory of Infectious Diseases, NIAID
- Dr. Fred M. Davenport, University of Michigan
- Dr. W. McD. Hammon, University of Pittsburgh
- Dr. Edwin H. Lennette, California State Department of Public Health
- Dr. Alexis Shelokov, Laboratory of Tropical Virology, NIAID

9. This Institute supported, during the past calendar year, two international conferences, one on newer respiratory viruses and the other on measles immunization; and a conference of immunologists. It is our conviction that creating such opportunities for group discussion of currently important health issues by recognized leaders constitutes one of the more productive means of expediting and extending intelligence in areas of newly emerging knowledge.

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EXTRAMURAL PROGRAM

Summary. 1

Annual Report of Program Activities

National Institute of Allergy and Infectious Diseases
January-December 1961

Extramural Programs

The year 1961 marks the tenth anniversary of the extramural program of the Institute. During these years--a decade of progress--the Institute has continued to promote an ever-increasing program of research and training. It has consistently worked to achieve the essential research objectives outlined ten years ago: to understand the fundamental nature of pathogens and allergens; to determine their mode of transmission; and to discover means of prevention and specific therapy.

Research Grants

The distribution of research funds is shown in Tables I and II. Data regarding the review and approval of research grants are given in Table III.

Allergy and Immunology

The realization of the need for basic investigations into fundamental phenomena underlying allergic and immune reactions has resulted in the advance of this Institute's grant-supported program in this area from 9 projects in 1956 ($78,258) to its present level of 282 grants, amounting to $4,987,462. In addition to the basic and clinical investigations carried out in universities and research organizations, there is a well-developed program for the identification, production and biological testing of purified antigenic fractions of allergenic substances.

Tropical Medicine and Parasitology

Basic investigations, involving many diciplines (immunology, physiology, biochemistry, etc.) have given us greater insight into the fundamental nature of parasitism and host-parasite relationships. These studies have steadily directed the progress of medical science toward the understanding of disease at the cellular and subcellular levels. Clinical studies have developed improved diagnosis, management and treatment of the large number of diseases caused by animal and plant parasites. This Institute supported 477 grants ($6,735,113) in tropical medicine, parasitology and zoonoses during 1961.

Staphylococcus

An alarming situation, especially in hospitals, has been developing in this country and abroad. Certain strains of staphylococcus, characterized by high degrees of virulence, communicability, and antibiotic resistance, actually have become more prevalent with the widespread use of penicillin and other antibiotics. The Institute is supporting a significant number of research projects, particularly with regard to hospital design and the use of

ultraviolet radiation and other measures to reduce the spread of infection. In 1961 the Institute supported 106 staphylococcus research grants totaling $2,079,800.

Virology

The problems of identification and discovery of heretofore unknown viruses in this rapidly expanding field are assuming unusual significance. Research grants awarded during 1961, exclusive of vaccines, involved 269 grants worth $6,388,716. Studies on acute viral respiratory diseases accounted for the major portion of this support.

As a consequence of the Council's interest and positive action based on a recommendation of the Virology and Rickettsiology Study Section, an Adenovirus Committee was established early in 1960 to give needed leadership for research on the adenovirus group of filtrable agents by providing a generally-accepted set of standards on which identification of these viruses can be based. The most urgent of the Committee's objectives has been the production of standardized reagents which would permit research workers to identify adenovirus strains. Through a technical group, the Production-Protocol Subcommittee, specifications were developed for the production of type-specific antisera for the known adenovirus types. Proposals solicited from several prospective producers were evaluated by the Subcommittee, the Adenovirus Committee, and the Council. On the advice of these groups, a contract and a special research grant have been awarded for the production, standardization, packaging, and certification of five-liter quantities of specific antisera for all 37 known adenovirus types, plus smaller quantities of prototype viral antigens. The first of these reagents will become available early in 1962. It is estimated that two years will be required to complete the program.

In October, the Institute was assigned general responsibility to centralize and coordinate the NIH activities related to the production of standardized viral reagents. A program therefore has been developed which embraces not only the work of the Adenovirus Committee, but also that of the Enterovirus Committee heretofore sponsored by the National Cancer Institute. Moreover, the scope is being expanded to include additional respiratory viruses, the arthropod-borne viruses, and miscellaneous viral agents not previously considered. Both contracts and grants will be used to meet two distinct needs: reference reagents for use of standards of comparison in research laboratories; and diagnostic reagents to implement screening studies as part of epidemiologic or other investigations.

Guidance for the program will be provided by a Board for Viral Reference Reagents, constituted of non-governmental consultants plus staff representatives of each interested Institute within the National Institutes of Health. Several technical panels will provide expert advice to the Board. The Adenovirus Committee has been reorganized as the Panel for Respiratory and Related Viruses. The former Enterovirus Committee has become the Panel for Enteroviruses. In addition, a Panel for Arthropod-Borne Viruses is being proposed. It is anticipated that this activity will provide comprehensive leadership in coordinating programs for the development of reference standards, including serologic reagents, for the entire field of virology. Close liaison

is being maintained with the Communicable Disease Center and with international organizations having related interests.

The Committee on Influenza Research, established by the Council, has been developing a valuable program of interepidemic research. It is felt that continuing research programs may well contribute more to the knowledge of influenza than the "emergency" research dictated by epidemics. Joint investigative studies encompassing epidemiology, virology, immunology, and pathophysiology of influenza are being encouraged.

In September, a Symposium on the Cardiopulmonary Physiology of Influenza was held at NIH to explore and define areas in which additional research should be encouraged. Some 50 university researchers attended the session, and a number made specific inquiries regarding Special Influenza Research Grants. The Council has recommended that FY 62 reserve funds for influenza research be increased from $175,000 to $200,000 (including $10,000 for support of the Committee's scientific evaluation activities).

The Surgeon General's Advisory Committee on Influenza Control accepted the Committee's recommendation regarding the dosage schedule for influenza vaccination, and the text of the official Influenza Fact Sheet was accordingly revised in October.

The Annotated Bibliography of Influenza by the American Institute of Biological Sciences is assured for five more years through a supplement to the original grant. The Bibliography has been favorably received and its 1,000 copies are widely distributed.

Conferences

Recognizing that exchange of information and critical discussion are basic to research progress, the Council continued to support research conferences in areas of special needs. Four conferences were supported during 1961: (1) International Symposium on Newer Respiratory Viruses; (2) International Conference on Measles Immunization; (3) Symposium on Opportunistic Fungi; and (4) a conference on The Relationship Between Asthma and Chronic Pulmonary Disease. In addition, support was recommended for travel of members of the Executive Committee planning the Second International Conference on Trichinosis and for some participants in a Symposium on Metabolism of Animal Parasites. Financial assistance was also given to the Carnegie Institute of Washington to bring ten foreign scientists to the Gordon Conference on Bacterial Cytology and to the Rockefeller Institute to insure U. S. representation at the First International Conference of Protozoologists in Prague.

Support of Research in Institutions Outside the U.S.A.

This Institute has a major continuing interest and responsibility to support research on diseases endemic to geographic areas outside the U. S. as a measure of self protection as well as to foster better preventive medicine abroad. During 1961, the Council awarded 69 grants to scientists at foreign institutions, a total expenditure of approximately $1,411,882. All

foreign grants must meet criteria established by the Internation Health Research Act of 1960.

Expeditious Council Actions

The Council has always taken special interest and positive action in certain research "emergencies." Recently the pertussis component of the quadruple vaccine (pertussis, diphteria, tetanus, and polio) has shown substandard potency. Immediate study of this important problem in pediatric immunization was approved by the Council. In another area, pilot studies showed that the simultaneous administration of attenuated measles vaccine and gamma globulin markedly reduces the incidence and severity of undesirable reactions. The Council approved support for an immediate field study in time to take advantage of the Spring measles epidemic.

Revisions in Review Procedures

The Institute, as part of an NIH-wide program has revised its policy regarding review of budgets for committed continuation years of research grant support. Applicants are now asked to present a detailed budget for the upcoming year's support, along with an estimate of the current year's expenditures, and summary progress report. These applications are reviewed to determine whether the full amount previously recommended is justified.

International Centers for Medical Research and Training

Under the International Health Act of 1960, designed to "advance the international status of the health sciences through cooperative enterprises," a fifth grant was awarded to Louisiana State University. This will support collaborative studies in New Orleans and Costa Rica, with the International Center in Costa Rica affiliated with a new medical school being started in San Jose. The grant is for a period of five years at an average expenditure of $400,000 per year.

In view of the current investment in the International Centers, a careful review of the five existing programs was made late in 1961. This analysis, together with the review of the Advisory Committee in January 1962 will serve as a basis for future planning.

Training Grants

This Institute has continued to develop its training grants program along lines designed to emphasize disease-oriented interests in allergy and infectious disorders.

The two training grant committees (the Allergy and Immunology Training Grant Committee and the Infectious Diseases and Tropical Medicine Training Grant Committee) have supplied highly competent professional review of applications assigned to this Institute.

Table IV indicates the distribution of training grant funds in support of the Institute's objectives. Table V gives data regarding review and approval of applications during the calendar year.

Certain foreign institutions have opportunities for providing outstanding training in special research areas to American students as well as their own nationals. Recognizing this fact the Institute currently is supporting two training grants at a Canadian institution, with the proviso that stipends from the grant be awarded to American citizens only. Three other training grant applications from foreign institutions are pending review.

Research Fellowships

Table VI shows the distribution of awards during the year, by areas of study and level of support. The data regarding review and approval of applications are presented in Table VII. The fellowship program of the Institute has followed the pattern set last year, with increasing emphasis on awards at the postdoctoral and special levels to produce increasing numbers of competent independent investigators.

The Special Fellowship Grant and the Senior Fellowship Grant programs were inaugurated in this Institute with the awarding of 15 Special Fellowship grants and 6 Senior Fellowship grants.

The above programs have been replaced with the Research Career Award Program. The Special Fellowship Grants and the Senior Fellowship Grants will be converted to the Research Career Development Award during the first year of the grant. The Career Research Professorship Grant Program will be converted to the Research Career Awards.

The Research Career Development Award is for the support of the promising young investigator, with at least three years of relevant research experience beyond the doctorate, who has demonstrated high potential for a research or academic career. The Research Career award is for support of the investigator who has demonstrated the ability to pursue a career in independent research. His research must have won the respect of scientific leaders in his field.

In order to provide initial support for the research of RCDA recipients, the Council has agreed that interim support (up to $5,000 per year) may be awarded on a year-to-year basis until the applicant receives either a corollary research grant or a regular research grant.

Highlights of Selected Accomplishment

During 1961, grantees have continued to report significant progress in the broad field of research activity encompassed in the extramural program. These accomplishments, primarily in basic research, serve as a foundation for future achievement in resolving specific clinical problems. The highlights which follow are representative of the scope of the program.

Recent reports from a grantee at the Gorgas Memorial Laboratory in Panama indicate that the bite of common forest sandflies may cause leishmaniasis or "forest yaws." For the first time, experimental infection in an animal (hamster) has been produced with cultures recovered from naturally infected sandfly species. Leishmaniasis, a protozoan infection, is widely distributed throughout Central and South American and is not infrequently seen in the United States in travelers from the Southern Hemisphere.

Grantees at the State University of Iowa have reported results of an immunization study which might lead to the development of an effective way to combat the Far East type of schistosomiasis, a widely prevalent parasitic disease. Rhesus monkeys inoculated with the non-human strain of Schistosoma japonicum and subsequently challenged with the human strain develop "rather strong" protection. With further research, the grantees hope that a state of absolute resistance to the challenge of infection might be reached.

The first canine schistosomiasis in the Western Hemisphere was reported by grantees at Tulane University. A search for the cause of severe dermatitis in Louisiana led the investigators to examine snails, birds, and mammals from the Mississippi River delta and marshy, coastal areas of Louisiana. They found Heterobilharzia americana, a species of schistosome, in racoons, nutrias, and dogs, indicating a wide range of hosts. The heterobilharziasis found in dogs is the first canine schistosomiasis recorded for the Western Hemisphere, and may prove to be an important disease of dogs in some parts of the southern United States. The extent to which H. americana would develop in man is not yet known; but it is possible that the cercariae, the free-swimming larvae, following the pattern of infection of other schistosomes infecting man could penetrate the skin and the schistomulae, the pre-adults, could invade the lungs and liver.

Grantees in Cincinnati found rhesus monkeys naturally infected with a plasmodium similar to the one which NIAID scientists found to be transmissible from monkeys to man. There has been wide belief that rhesus monkeys are free of naturally acquired malaria. Examination of 228 monkeys from Pakistan showed 22 to be naturally infected. The importance of these observations to investigators of plasmodial diseases is obvious; and workers using rhesus monkeys in other investigations should be alert to the existence and potential liabilities of concurrent malaria infections.

Suggestions that staphylococcus alpha toxin is a general cytoplasmic poison is disputed by grantees at the University of Minnesota. Alpha toxin, the powerful necrotizing agent produced by staphylococcus, is probably important in the pathogenesis of localized staphylococcal disease and the shock-like picture sometimes associated with staphylococcal septicemia. However,

its precise role in abscess formation is unclear, nor has it been clear whether it acts as a general cellular poison or selectively on the smooth muscle cells. Experiments demonstrated that epithelial cells in tissue culture and isolated preparations of living cardiac and skeletal muscles are resistant to the action of the toxin. On the other hand, studies show that smooth muscle, regardless of its origin, appears to be exquisitely sensitive to the toxin and is irreversibly damaged by its action in a few seconds. These studies may help clarify the complex interaction of the microorganism, its side products, and host.

Definite inhibition of bacterial species Bacillus subtilis and Staphylococcus aureus was achieved when cultures of fungal components of selected lichens were tested by grantees at Clark University. These biologically active compounds were extracts from 3 of 11 pure cultures of lichen mycobionts tested against various bacteria and fungi. Lichens themselves form many chemical compounds which possess strong antibiotic activity, but they are notoriously slow growing and not amenable to laboratory cultivation. The research possibilities on the physiology and chemistry of lichen fungi are limitless, considering that some 15,000 unique and diverse lichen species occur in nature and that the fruitful results of this study pertains to the mycobionts of only three of these.

The advantages of using fluorescent antibodies for the diagnosis of bacterial meningitis and acute streptococcal infections were described by grantees at the Child Research Center and Children's Hospital of Michigan. The immunofluorescence technique proved to be a sensitive, reliable and rapid method for early identification of Hemophilus influenzae and Group A Streptococci.

In a study at Harvard, grantees found a large percentage of cystic fibrosis patients originally misdiagnosed as respiratory allergy. They also demonstrated the possibility of masked, and therefore untreated respiratory allergy in CF patients. This study contradicts previous reports by other investigators that patients with respiratory allergy alone may also have elevated sweat-electrolyte levels. The grantees found the levels in these patients to be well within the normal range. It was pointed out, however, that parents of patients with cystic fibrosis, although presenting no clinical manifestation themselves, may manifest elevated sweat-electrolyte levels. This may be explained apparently on the basis of a carrier state for the gene of cystic fibrosis.

A study by grantees at Children's Hospital Medical Center, Boston, and Harvard University showed that a defect in the function of the eccrine sweat glands in infants can be detected by iontophoresis as early as one day of age. Establishment of preoperative diagnosis of cystic fibrosis by this method is helpful for surgical management of intestinal obstruction.

NIAID grantees at Children's Hospital, Philadelphia, reported use of a screening procedure which permits an evaluation of the effect of enzymes on tracheobronchial secretions from cystic fibrosis patients. Results showed that of ten test enzymes, pancreatic dornase acted most markedly on secretions responsible for pulmonary obstruction in CF patients. The investigators stress that the procedures they use should serve only as a guide in the selection of

potentially therapeutic agents because the factors involved in the clinical situation are more complex than those in the in vitro screening. Controlled clinical studies of the therapeutic use of pancreatic dornase in cystic fibrosis are currently under way. Until these have been analyzed, caution is advised in using dornase in routine treatment because of the many dangers associated with its promiscuous use.

NIAID grantees, in a recent epidemiologic study in California described an unusual childhood illness linked to Coxsackie virus, type A 16. For a number of years, herpangina has been recognized as a widely prevalent childhood disease. In 1957 an outbreak of illness similar to herpangina but differing in several significant aspects occurred in Toronto, Canada, and was linked to Coxsackie virus, type A 16. It remained unique until 1959 when a disease with an apparently identical syndrome was rediscovered in various localities in California. The syndrome was later observed in all major geographic areas of the State, and the California State Department of Health estimated a total incidence running into the thousands. The illness is not known to have been recorded previously in the United States, and the experience with Coxsackie A 16 provides a notable example, in the brief history of enterovirus infections, of the sudden emergence of one virus type in an outcropping of clinical illness in one geographic area, followed by a return to obscurity for a variable time before unpredictably reappearing in some distant locality.

The cause of acute viral infections of the lower respiratory tract has been a matter of considerable speculation and is the subject of a continuing study by grantees at the University of Wisconsin. In a 7-year study, viruses were shown to cause between 90 and 95 percent of acute infections of the lungs and bronchi of hospitalized young adults. Nearly 25 percent of the patients had infections associated with the Eaton agent, the most impressive association found in the study.

Although rapid antibody response often makes the neutralization test of doubtful value in diagnosing many viral infections because of the "recall phenomenon," results of a large study by grantees in California have now confirmed the diagnostic value of the recently developed colorimetric (metabolic inhibition) procedure for assaying neutralizing antibodies to Coxsackie group B viruses. This new test should become a valuable adjunct to virus isolations and for diagnosing infections by this group of agents.

Grantees at the Children's Hospital Medical Center, Boston, reported results of small trials of attenuated measles vaccine prepared by them. Antibody development occurred in 96 percent of children regarded as susceptible to measles. In a significant number of cases, none of the vaccinated children have developed measles following natural exposure to the disease.

In another study at the Center, grantees examined materials from a large sampling of the cases of paralytic poliomyelitis occurring in Massachusetts in 1959. Poliovirus III was found in all but one case. It appeared that little protection was afforded those who had previously received Salk vaccine.

Grantees at the University of Miami have reported that normal adult serum shows marked ability to retard the *in vitro* growth and reproduction of *Candida albicans*. This inhibitory complex is greatly diminished in patients with acute blood dyscrasias, but essentially unaffected in some malignant states. A major problem is the identification of the components which endow the serum with its capacity for fungistasis. The investigators believe that the inhibition may be related, at least in part, to the nutritional soundness or to metabolic competency. The high inhibitory activity of sera from terminal cases of cancer, however, argues against the concept that a debilitated state *per se* predisposes an individual to fungal overgrowth.

Grantees at Notre Dame University, carrying on genetic studies, indicated that an inherited factor is the cause of a predominance of males in certain strains and in progeny of the *Aedes aegypti* mosquito. This factor appears to be transmitted only by males and is not due to differential mortality, at least in postgametic stages. No factor causing abnormal sex ratio has been reported previously for mosquitoes. Mass release of male-producing males might be used in mosquito control operations.

An effective method for disinfecting vegetables has been reported by a Tulane grantee. In regions of the world where human feces are used for fertilizer, vegetables may be contaminated simultaneously with infective stages of Ascaris, hookworms, and other helminths. Although a number of chemicals kill such parasites, no agent has been acceptable for use as a disinfectant of fresh vegetables. The investigator found that a solution containing 70 ppm free iodine, prepared by dissolving commercially prepared tablets in distilled water, quickly kills common intestinal nematodes and does not alter the flavor or appearance of fruit and vegetables.

TABLE I -- RESEARCH GRANTS PROGRAM (NIAID)
(FISCAL YEARS)

Area of Support	'59 Appropriation* $14,880,000		'60 Appropriation*** $21,593,000		'61 Appropriation **** $28,951,000		'62 Appropriation $36,725,000 (Estimated)	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Allergy	107	$1,550,824	134	$2,184,636	155	$2,924,330	182	$3,202,048
Immunology	77	1,033,864	116	1,910,816	127	2,215,733	141	2,477,05
Bacteriology	280	3,791,288	378	5,613,529	428	7,152,883	450	7,884,288
Virology	173	3,426,721	233	4,794,512	269	6,388,716	350	7,038,464
All Others (Antibiotics, Arthropods, Biochemistry, Epidemiology, Genetics, Mycology, Parasitology, etc.)	492	5,085,583	550	6,410,413	636	8,730,556	600	9,606,144
Grand Total	1,129	$14,888,280**	1,411	$20,913,906**	1,615	$27,412,218**	1,723	$30,208,000

* The '59 appropriation included $150,000 for Gorgas Memorial Laboratory and $18,000 and $39,000 respectively for Russian Translation Service. In addition, $442,000 was deducted from the '59 appropriation to cover NIAID salary increases.

**Excess listed over appropriation represents extensions without additional funds and grants, paid from previous year fiscal funds, which terminate during the indicated fiscal year.

***The '60 appropriation included $500,000 for metabolic (clinical) centers, $150,000 for Gorgas Memorial Laboratory, $44,000 for Russian Translation Service, $1,000 for the CDC Diagnostic Reagents Program, and $41,200 for the Children's Bureau project on cystic fibrosis.

****The '61 appropriation included $750,000 for Gorgas Memorial Laboratory, $500,000 for Clinical Research Units, $500,000 for International Medical Research. In addition, $140,000 was used for Senior Fellowship, Special Fellowship, and Career Research Professor Programs.

TABLE II -- RESEARCH GRANTS PROGRAM (NIAID) SPECIAL AREAS 1/

Area of Support	Fiscal Year 1959		Fiscal Year 1960		Fiscal Year 1961		Fiscal Year 1962	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount (Estimated)
1. Acute Respiratory Disease 2/	71	$1,428,586	119	$2,290,187	126	$2,972,458	142	$3,269,714
2. Cystic Fibrosis	23	382,539	19 3/	336,835	9	240,115	11	255,197
3. Tropical Medicine and Parasitology								
Total	331	3,229,304	420	5,105,550	477	6,735,113	541	7,416,668
Parasitology:								
Helminths	78	696,375	101	1,010,601	117	1,391,110	132	1,530,991
Protozoa	76	762,524	82	945,966	96	1,319,643	109	1,450,413
Arthropods	75	653,187	86	890,011	100	1,273,057	113	1,410,124
Arthropod-borne Viral Diseases:								
Dengue	4	69,339	4	89,383	4	79,352	5	87,293
Encephalitis	7	135,046	13	316,625	19	542,506	22	597,623
Yellow Fever	1	27,500	1	25,000	2	30,900	2	33,574
Non-Parasitic Tropical Diseases of Importance:								
Cholera	2	19,165	4	54,259	4	75,924	5	87,293
Leprosy	7	106,297	7	116,681	9	210,482	10	228,305
Salmonellosis	8	96,586	14	171,236	11	157,179	13	167,871
Shigellosis	3	70,726	2	85,250	4	70,878	5	80,578
Fungal Diseases	70	592,559	78	868,312	79	949,172	89	1,040,805
4. Staphylococci:	59	1,002,566	84	1,541,342	106	2,079,800	121	2,296,775
5. Tuberculosis and related areas:	73	995,618	94	1,519,821	106	1,777,663	119	1,945,878
6. Enteroviruses(excluding poliomyelitis)	9	166,730	11	245,045	15	282,677	17	318,996
7. Urinary Tract Infections (pyelonephritis)	5	108,578	12	191,000	19	403,195	22	446,596

1/ Included in areas listed in Table I.

3/ Includes $41,200 for Children's Bureau project.

2/ Includes Airborne Allergy, Adenoviruses, Influenza, Ornithosis, Pneumococci, Q Fever and Viral Pneumonia

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2/ Includes Airborne Allergy, Adenoviruses, Influenza, Ornithosis, Pneumococci, Q Fever and Viral Pneumonia

TABLE III

RESEARCH GRANTS REVIEWED DURING CALENDAR YEAR 1961

	Received		Approved	
March	No.	Amount	No.	Amount
New	247	$6,273,216	132	$3,164,540
Continuations	112	1,910,824	95	1,499,790
	359	8,184,040	227	4,664,330
June				
New	218	5,516,853	124	3,007,582
Continuations	1,371*	23,113,302	1,322	22,221,166
	1,589	28,630,155	1,446	25,228,748
November				
New	207	4,513,901	98	2,008,642
Continuations	110	2,436,112	87	1,911,178
	317	6,950,013	185	3,919,820

*Includes continuation grants for which previously recommended support was reaffirmed (1,166 for $19,172,249).

TABLE IV

TRAINING GRANT PROGRAM (FISCAL YEARS)

Discipline	'59 Appropriation $1,787,000 No.	'59 Appropriation $1,787,000 Amount	'60 Appropriation $3,621,000 No.	'60 Appropriation $3,621,000 Amount	'61 Appropriation $4,790,000 No.	'61 Appropriation $4,790,000 Amount	'62 Appropriation $5,400,000 (Est.) No.	'62 Appropriation $5,400,000 (Est.) Amount
Allergy and Immunology	19	$525,203	30	$1,293,010	37	$1,284,215	44	$1,547,804
Infectious Diseases	23	793,830	34	1,449,651	53	2,073,273	63	2,425,134
Tropical Medicine and Parasitology	18	439,668	22	807,593	27	985,245	33	1,049,179
	60	$1,758,701 1/	86	$3,550,254 1/	117	$4,342,733 2/	140	$5,022,117

1/ Chairman's grants are not included in these figures.

2/ This figure does not include $397,734 that was used to forward finance 17 grants to a July 1 beginning date, nor does it include $34,500 for Scientific Evaluation Committee Grants.

TABLE V

TRAINING GRANTS REVIEWED DURING CALENDAR YEAR 1961[1]

	Received		Approved	
March	No.	Amount	No.	Amount
New	16	$440,695	12	$278,881
Continuations	0	--	0	--
	16	$440,695	*12	$278,881
June				
New	14	$601,667	12	$480,082
Continuations**	108	$3,626,893	108	$3,626,893
	122	$4,228,560	120	$4,106,975
November				
New	19	$ 668,417	13	$ 443,022
Continuations	0	--	0	--
	***19	$ 668,417	13	$ 443,022

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[1] Amounts do not include $50,000 approved for Scientific Evaluation Committee grants, nor $395,866 needed to forward finance 17 grants.

* Includes 2 for $60,170 deferred from previous Council.

** Includes continuation grants for which previously recommended support was reaffirmed.

*** Includes 3 for $123,621 deferred from previous Councils and not included in any of the entries above.

TABLE VI

RESEARCH FELLOWSHIPS PROGRAM (NIAID)
(FISCAL YEAR 1961)*

	POSTDOCTORAL				SPECIAL				CAREER DEVELOPMENT GRANTS			
									(Special)		(Senior)	
	New		Continuation		New		Continuation		New		New	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Allergy	10	$62,137	7	$51,600	4	$41,553	--	$------	2	$32,557	3	$56,646
Antibiotics	4	28,235	--	------	--	------	--	------	--	------	1	12,533
Arthropods	--	------	2	13,079	--	------	--	------	--	------	--	------
Bacteriology	5	29,446	6	43,500	2	43,500	--	------	--	------	1	20,028
Biochemistry	9	48,620	14	86,532	3	13,470	--	------	--	------	1	12,247
Detection	1	6,716	--	------	--	------	--	------	--	------	--	------
Epidemiology	3	25,479	--	------	--	------	--	------	--	------	--	------
Fungi	1	7,000	--	------	1	10,240	--	------	--	------	--	------
Genetics	1	6,615	3	17,000	--	------	--	------	--	------	--	------
Helminthology	4	26,364	4	21,000	1	9,191	--	------	--	------	--	------
Immunology	10	63,946	5	30,112	1	8,130	--	------	1	10,773	--	------
Physiology	--	------	1	3,835	--	------	--	------	--	------	--	------
Protozoology	1	5,041	1	5,000	--	------	--	------	--	------	--	------
Rickettsiology	--	------	1	7,000	--	------	1	6,382	--	------	--	------
Spirochetology	--	------	1	8,860	--	------	--	------	--	------	--	------
Virology	21	135,755	6	43,939	6	55,267	--	------	4	62,951	--	------
Degenerative Diseases	8	51,091	--	------	--	------	--	------	--	------	--	------
Undetermined	3	18,630	1	6,000	--	------	--	------	1	4,808	--	------
	81	$515,042	52	$337,457	52	$161,891	1	$ 6,382	8	$111,089	6	$101,454

*Fellowships Awarded

TABLE VII

RESEARCH FELLOWSHIPS REVIEWED DURING FISCAL YEAR 1961

	New	Continuations
Postdoctoral	123	28
Special	36	2
Research Career Development Awards		
Formerly Special Fellowship Grants	17	
Formerly Senior Fellowship Grants	12	
Research Career Award		
Formerly Career Professorship	16	

ASSOCIATE DIRECTOR IN CHARGE OF RESEARCH

INTRAMURAL RESEARCH PROGRAM

Summary. 1

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
ANNUAL REPORT OF THE ASSOCIATE DIRECTOR IN CHARGE OF RESEARCH

INTRAMURAL RESEARCH PROGRAM
CALENDAR YEAR 1961

The direct research activities benefited this year by availability of additional space in Buildings 5 and 8 and renovation of a number of laboratory areas. Next year will see additional space readjustment which will permit more adequate utilization of staff resources. The staff of the Laboratory of Biology of Viruses moved into new quarters on the third floor of Building 5, releasing additional space in Building 7 for expanded work in acute respiratory viral diseases. Additional space is also available for the Laboratory of Parasitic Diseases and Laboratory of Parasite Chemotherapy in Building 5. The Laboratory of Tropical Virology occupied enlarged laboratories renovated for both highly infectious agents and for ordinary virologic procedures.

After twenty-five years of distinguished research in the Public Health Service, Dr. Harry Eagle retired to accept an academic position. The Laboratory of Cell Biology of which he was Chief was incorporated into the Laboratory of Biology of Viruses under Dr. Karl Habel as a Section. It remains essentially intact and continues productive studies in cell biology.

The Laboratory of Germfree Animal Research established a new Experimental Pathology Section under Dr. Edwin Lerner. This Section will support ongoing studies in germfree life and also provide histopathologic consultation to NIAID investigators.

The Laboratory of Tropical Virology in which the Middle America Research Unit is administratively located secured expanded space in Bethesda, as well as improved facilities in the laboratory in the Panama Canal Zone. Dr. Alexis Shelokov, Chief of the Laboratory, returned to Bethesda in July and relinquished his duties as Director, MARU to Dr. Henry K. Beye formerly with the Laboratory of Clinical Investigation. An Epidemiology Section was established in MARU in addition to other previously established sections.

On his return from a year of study in Europe, Dr. Carl L. Larson was assigned to the Office of the Director, NIAID, to conduct an evaluation of the total NIAID research program emphasizing particularly the extramural training program. Dr. Cornelius B. Philip succeeded Dr. Larson as Director of the Rocky Mountain Laboratory and Dr. Herbert Stoenner became Assistant Director. Dr. William Hadlow rejoined the staff of RML as Head of the Pathology Section. Plans were initiated for the construction of an animal house and insectary with new funds provided by the Congress.

Dr. Don Eyles of the Laboratory of Parasite Chemotherapy established a field station in Kuala Lumpur, Malaya on the grounds of the Medical Research Institute to investigate more intensively the problem of simian malaria. He is assisted by two NIAID staff members and local technical help.

1

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Although the position of Chief, Laboratory of Immunology has not been filled, research work of that laboratory is proceeding successfully under the able guidance of Dr. John Tobie, Acting Chief. One of the successful ventures of the Laboratory of Immunology which influences work throughout the NIH is the series of weekly seminars in immunologic subjects by staff members or outside scientists. A highlight was a special seminar in honor of the late Dr. Jules Freund, the first Chief of the Laboratory of Immunology, presented in December by Dr. Merrill Chase.

As in the past, the Institute participated in the NIH Research Associate Program. Recent medical graduates who can fulfill their military duty by a commission in the Public Health Service are selected and assigned to research laboratories. Seminars and lectures provide a scope of interest but the principal value is in actual research experience under the guidance of a preceptor. This is the fifth year of the program. It is already clear that these young medical scientists form an important reservoir of talent for the staff of this Institute and other research centers in universities and medical schools.

As a part of the United States effort to establish better scientific communication with the USSR, this Institute sponsored the U. S. Infectious Disease and Microbiology Exchange Mission. A group of well known virologists, headed by Dr. Robert J. Huebner, Chief, Laboratory of Infectious Diseases, NIAID visited research institutions and laboratories in Russia for four weeks in the spring. Members of the delegation were:

Dr. Robert M. Chanock, Laboratory of Infectious Diseases
Dr. Fred M. Davenport, University of Michigan
Dr. W. McD. Hammon, University of Pittsburgh
Dr. Edwin H. Lennette, California State of Public Health
Dr. Alexis Shelokov, Laboratory of Tropical Virology

As a further step in the international exchange of scientific information, this Institute was host to Dr. Nicolai Petrovich Yelinov, Deputy Director of the Leningrad Chemical-Pharmaceutical Institute, from September until December. Dr. Yelinov worked in the laboratory of Dr. Chester Emmons, investigating mycotic diseases. In turn, Dr. Jacob A. Brody of the Laboratory of Tropical Virology left for the USSR in December to spend six months in the laboratory of Professor M. P. Chumakov, Director, Institute of Poliomyelitis and Viral Encephalitis, Academy of Medical Sciences, Moscow. Six staff scientists of this Institute attended the Fifth International Biochemical Congress, Moscow, during August.

The Institute has continued to assign carefully selected staff scientists to other research laboratories either to pursue their own line of investigation or to learn new concepts and techniques. Dr. Eugene C. Weinbach, Laboratory of Parasitic Diseases is working at the Wenner-Grens Institute, University of Stockholm, Sweden. Dr. Philip McMaster, Laboratory of Immunology is at Pasteur Institute, Paris. Dr. Maryjane K. Cook, Laboratory of Infectious Diseases continues her studies at Max-Planck Institut für Virusforchung, Tubingen, Germany. Dr. Kelsey C. Milner, Rocky Mountain Laboratory, began work at The Karolinska Institut, Stockholm, Sweden this fall.

Dr. Sanford Stone started a year's work at the Hopital Broussais, Paris. Dr. J. Frederick Bell was awarded a Guggenheim Fellowship which provided him an opportunity to visit the research laboratories in Middle Europe interested in tularemia. In December, Dr. William J. Jellison was assigned to the Pan American Sanitary Bureau to conduct investigations at the Zoonoses Center, Azul, Argentina.

Once again the Institute was host to many scientific guest workers who remained from several days to several months. Five foreign scientists joined the Institute in the Visiting Scientist program to engage actively in current investigations. Six scientists supported by Fellowships worked with members of the Institute staff.

Each year there is increasing demand and need for training opportunities at the Institute. Approximately 25 Visiting Scientists, Fellows, Research Associates, and Clinical Associates at the postdoctoral level engaged in research activities in a training capacity. It is clear that the various Institutes at NIH have the opportunity to play a major role in the post-doctoral degree education of biomedical scientists in this country. It is a role which this Institute can pursue effectively, because of its resources and interest in a wide variety of research problems in infectious and allergic diseases.

A long felt need in the study of viruses as a cause of disease is a readily available supply of uniform reference antisera and antigens. Virologists find that with the increasing number of isolates (now numbering in the hundreds) their investigations are curtailed by lack of identifying reagents. To fill this need, the Director, NIH requested this Institute to establish a program under guidance from representatives of interested Institute Councils, and staff. This effort extends the support which the National Cancer Institute already has given for reagents of the enteroviruses and this Institute for the adenoviruses. Panels of experts were established for enteroviruses and adenoviruses and will be established for the arthropod-borne viruses. They will write specifications for reagents and determine procedures for monitoring the quality of the material produced under contract. Although this is supported primarily by extramural funds, responsibility for it rests with the Associate Director in Charge of Research.

As new laboratory methods for uncovering viruses are developed, and their role as a cause of acute respiratory disease determined, it has become clear that vaccines for immunization will be needed as soon as possible. This Institute has initiated a Vaccine Development Program which is intended to fill the gap between the fundamental research conducted in laboratories and the production of tested prophylactic vaccines suitable for use by practicing physicians. The rapid advancement of research accomplishments in respiratory diseases demands equal effort to translate these into practical usefulness. The Institute will be advised by a Board of outside scientists and staff members on specific developmental problems and the particular competence of various laboratories to fulfill the project requirements.

The following pages describe the accomplishments of scientists working in Institute laboratories. They comprise a wide spectrum of research endeavor. All projects have been significant and contributed in various measure to scientific knowledge and to research techniques. Of special interest has been the development of procedures for utilizing inmate volunteers in the studies of minor respiratory illnesses and malaria. The excellent clinical facilities at the Clinical Center and the carefully controlled laboratory procedures insured a high degree of precise clinical measurement and safety. In a short time with relatively little cost the project has given valuable information which otherwise would have been impossible or inordinately time consuming and expensive.

Advancements which are particularly timely and influential are: the demonstration that the Eaton agent, an important cause of respiratory illnesses, is a pleuropneumonia-like-organism; the description of the first cases of Venezuelan equine encephalitis in Central America; the axenic culture of Entamoeba histolytica; demonstration by immunofluorescence of antibody production in human malaria; and the evidence for a new "foreign" cell antigen produced by polyoma virus as it transforms normal cells to tumor cells. Other equally important findings are included in the following report of activities.

LABORATORY OF CLINICAL INVESTIGATION

The program of the Laboratory has been enlarged by the development of projects in viral respiratory disease, immunology, mechanism of fever, and malaria, without sacrifice of any previous activity. In contrast to previous years when optimum bed occupancy was achieved only with difficulty, the daily census has been maintained near 100 percent by reason of the volunteer program, and there is now a waiting list for other cases which permits a more careful selection of those to be admitted.

VIRAL INFECTION OF VOLUNTEERS

The largest project of the laboratory has been the study of viral respiratory disease in prisoner volunteers. During the year, the organization and function of this activity has become more efficient. A total of 189 volunteers have been inoculated with one of 14 different respiratory viruses or the Eaton agent. The principal purpose of these studies has been to determine the capacity of the agents to cause illness and to study the virological and immunologic response to inoculation. The viruses tested were, with exception of influenza, suspected of causing significant amounts of respiratory viral infection, but their role had not been defined. Thus, a determination of their capacity to cause illness represented an important step in assessing their role in the total picture of respiratory disease in the general population.

A classical type of moderately severe viral influenza has been produced in susceptible volunteers while a milder disease or no illness occurred in those with specific antibody. Illness and viral excretion began by the second day after inoculation and virus was still present in some cases 5 or 6 days later, and after recovery from illness. These studies have demonstrated a type of infection and illness which lends itself to many kinds of clinical study.

Despite many close biologic and biochemical similarities it was found that Coxsackie A-21 (the Coe agent) caused an acute, febrile flu-like illness while Coxsackie A-24 (the Pett virus) caused no illness. Virus was repeatedly isolated from the throat of volunteers given A-21, while A-24 was found only in rectal swabs. Several higher-type adenoviruses have been shown to produce disease when inoculated by cotton swab into the conjunctiva beneath the lower lid but not when given intranasally or by spray into the throat. In all situations, however, viral infection occurred with both throat and anal swabs positive. The higher type agents appear to be less virulent than lower type adenoviruses, as judged by comparison with earlier studies.

Reo-viruses obtained from human sources failed to produce illness although viral infection occurred in susceptibles. Recently, a common cold syndrome with rhinorrhea, malaise, and in some cases, fever, has been produced by three new and presently unclassified enteroviruses. The incubation period was only a few hours.

A joint venture is currently underway with investigators at Ft. Detrick, Maryland. They are preparing several viral agents in small particle aerosol and with their equipment, cautious studies of the effect of introducing inocula of small particles deep into the bronchial tree will be made. It is also planned to test the effect of an adenovirus inhibitor of biologic origin in treatment of infection. (This is distinct from interferon, and there is some prospect for future studies of this substance as well.)

PENICILLIN DERIVATIVES Two new penicillin derivatives have been tested in staphylococcal infection. The agents, Prostaphlin, manufactured by Bristol Laboratories, and SKF No. 12141, manufactured by Smith, Kline & French, are similar in that they are substantially resistant to destruction by penicillinase, they are significantly more active on a weight basis against sensitive staphylococci than dimethoxy-phenylpenicillin, the first of the penicillinase resistant compounds (see last year's report), and they are active after oral administration. Limited clinical studies have revealed that these agents are effective in the treatment of staphylococcal infection, including those resistant to older forms of penicillin. Biochemical studies on these compounds have also been made and will be described elsewhere in the report.

SALMONELLA CARRIERS Studies were made of 29 chronic salmonella carriers and it was revealed that gallstones present in every case contained salmonella (most were S. typhosa). When these stones were incubated in high concentrations of antibiotics, salmonella persisted and grew in subsequent cultures not containing antibiotics. Consistent with these findings was the observation that antibiotic treatment alone was not effective in curing the carrier state. On a basis not yet fully explained, was the further observation that cholecystectomy alone was often not effective in curing carriers, suggesting infection in sites other than gallstones which were the basis for perpetuation of the carrier state (a few instances of positive cultures from liver tissue were found). Finally, it was found that lasting cure occurred if antibiotic treatment was combined with cholecystectomy. It was estimated that over 300 cases and 3 deaths from typhoid occurred in contacts of this group of patients.

MALARIA IN VOLUNTEERS Following demonstration of human infection with the simian vivax-type malaria, Plasmodium cynomolgi bastianellii, several prisoner volunteers infected with this agent, and others inoculated with human vivax strains were studied at the Clinical Center. In addition to the usual parasitologic determinations, radioactive chromium survival tests, excretion of adrenal steroid metabolites, liver function, a number of other parameters were measured. Among other findings was the observation that a significant reduction in serum haptoglobin concentration took place in association with hemolysis of the red blood cells.

The most significant observation, however, was the demonstration of fluorescent stainable malaria antibody appearing approximately two weeks after onset of illness and reaching a peak about one month later. Titers as high as 1/5120 were frequently reached. Following recovery the titers slowly decreased. In connection with these studies, it was found that the concentration of circulating gamma globulin in the malaria patients increased

during infection, and the highest values of gamma globulin coincided closely in time with the occurrence of the highest titer of malaria antibody.

MECHANISMS OF FEVER A new program begun this year has been that of investigation of host response to fever, and causes of fever and related phenomena in certain febrile diseases of obscure origin. The studies are presently centered around the disease known as familial Mediterranean periodic fever or familial polyserositis. It is an inherited disease of certain persons of Jewish, Armenian and sometimes of Turkish descent. It is characterized by many years of progressive, disabling attacks of non-infectious fever and peritonitis unrelieved by any presently known treatment. Studies here have been concerned with the effect of the recurrent fever on mechanisms of heat loss when the patients are stressed in a low temperature chamber. It appears now that patients with polyserositis have lost some responsiveness to cold stimulation as compared with normals. Studies of gastrointestinal motility, metabolism of adrenal steroids, fibrinogen and platelets, and of the effect of endotoxin injection are underway.

FUNGUS DISEASE The program in systemic fungus infection has continued in a vigorous way. A number of clinical reports have been made of treatment of various kinds of fungus disease with amphotericin and the new agent of Hoffman-La Roche, X5079C. Both drugs are extremely useful in treatment of fungus disease as described in the following table:

	Amphotericin	X5079C
Blastomycosis	Good	Good
Histoplasmosis	Good	Good
Sporotrichosis	Good	?Good
Cryptococcosis	Good	Doubtful
Coccidioidomycosis	Slight	Doubtful
Candidiasis	Moderate	Doubtful

Since amphotericin causes significant renal toxicity and local irritation, especially when introduced into the spinal canal these features of treatment have been studied. It was found that a persistent renal tubular lesion characterized by prolonged depression of para-amino hippurate clearance is caused by amphotericin. Furthermore, recent studies of biopsies of kidney from patients under treatment reveal necrosis of glomeruli. This is an unusual renal lesion and is under detailed investigation in dogs. So far no effective means has been found to control the irritative effects of the drug on the central nervous system after intrathecal instillation. The nerve damage encountered has been so severe that local use of the agent in meningitis (cryptococcal) will henceforth be administered only to those cases in which it is considered an absolute essential.

X5079C was found to cause a prompt rise in bromsulfalein retention despite only slight evidence of interference in other kinds of liver function. This received careful examination in dogs and the effect is now revealed to

be a competition of the antibiotic for the excretory route for bromsulfalein. The exact mechanism has not been determined. Prolonged treatment with the agent causes some liver damage but it appears to be sufficiently slight to permit therapeutic use of the drug.

PENICILLIN-RESISTANT STAPHYLOCOCCAL INFECTIONS

Work in the past three years on the problem of penicillin-resistant staphylococcal infections has developed into two distinct problems. The first phase of the investigation dealt with the nature of the resistance of staphylococci to penicillin. It was established that naturally-occurring pathogenic strains of *Staphylococcus aureus* which are resistant to penicillin, are resistant by virtue of their containing an enzyme, penicillinase, which rapidly destroys penicillin G. A series of papers have been published on the biochemical properties of this enzyme as it occurs in *S. aureus* and on its role in penicillin resistance.

The second phase of the investigation has been concerned with the many new penicillins that have become available in the past two years as the result of a technologic breakthrough in the field of penicillin chemistry; namely, the ready availability of 6-aminopenicillanic acid as the immediate precursor of a wide variety of penicillins previously unknown. When it was determined that resistance of staphylococci was based on their ability to destroy penicillin G at a rapid rate, it became evident that a possible solution to the problem would be to find a modification of penicillin which would be resistant to the destructive action of penicillinase and yet retain antibiotic activity. When 2, 6-dimethoxyphenylpenicillin was given to the Laboratory for clinical trial, it was very soon found that this new penicillin met the essential biochemical criteria and offered therapeutic promise. Our laboratory, by virtue of being the first to obtain *staphylococcal* penicillinase in large amounts in concentrated and purified form, was able to establish the absolute rate of destruction of the new penicillin by staphylococcal penicillinase and show it to be more resistant than penicillin G by a factor of about 200 to 1. The consistently good therapeutic results obtained with this drug on extensive clinical usage in the past year have tended to verify the conclusions drawn from the laboratory findings.

Similar techniques have enabled us to evaluate all the new penicillins which have been offered to us for clinical trial including an isoxazolyl penicillin which is not only resistant to penicillinase but is also sufficiently stable to acid to permit its oral use. Such a penicillin would have an advantage over 2,6-dimethoxyphenylpenicillin which is not effective after oral administration and must be given intramuscularly or intravenously.

CLINICAL IMMUNOLOGY

The development of a clinical immunology section has provided the Laboratory of Clinical Investigation with an increasing number of laboratory techniques to support our clinical studies. The immunoelectrophoresis technique, other gel diffusion methods, and the fluorescent antibody techniques have been used to study the serum of patients with the nephrotic syndrome, lupus erythematosus, and many other of the disease entities under study by other members of the laboratory.

A group of 60 patients with the nephrotic syndrome studied over the past eight years are being followed to evaluate the effect of steroid therapy in these diseases. Similar studies are being made with a group of patients with lupus erythematosus and in addition, these patients are being evaluated for the degree of skin test hypersensitivity to autologous leukocytes. Since the therapy with steroids is often not completely effective and since initial results suggest that those purine antimetabolites which apparently act by suppressing antibody production are effective in the treatment of certain diseases associated with auto-immune phenomena, a study of the effectiveness of these drugs in the nephrotic syndrome and lupus erythematosus has begun.

MOUSE PLASMA CELL TUMORS

Two years ago it was discovered in this laboratory that mice injected with Freund's adjuvant and certain antigens developed chronic ascites containing a high titer of antibody to the administered antigen. Later it was shown that a certain number of the animals, and this was strain-related, developed plasma cell tumors which were rapidly fatal. Further, it was observed that the tumor-bearing mice were poor producers of antibody. These tumors have been found to produce predominantly one or another types of globulins (alpha, beta, gamma) and currently studies are under way to measure the relative responsiveness to antigenic stimulation of mice with these tumors.

LABORATORY OF IMMUNOLOGY

PASSIVE TRANSFER OF ALLERGIC ENCEPHALOMYELITIS

The passive transfer of allergic encephalomyelitis was accomplished when lymph node cells from sensitized Strain 13 inbred guinea pigs were transferred to normal Strain 13 recipients. Furthermore, this experimental autoimmune disease may be transferred from sensitized adult Strain 13 guinea pigs to newborn Strain 13 animals. Strain 2 inbred guinea pigs are relatively resistant to allergic encephalomyelitis and the disease is not transferred to the F1 generation animals resulting from a Strain 2 - 13 cross. The F1 animals evidently inherit the Strain 2 resistance. Passive transfer with viable lymphoid cells represents an important tool in the study of immune phenomena. In histocompatible animals, the variable of transplantation immunity is eliminated, the transferred cells thrive, and the cells go on to immune maturity in the recipient. With the strategic use of Strain 2, Strain 13 and their F1 hybrids, crucial problems concerning the mechanism of hypersensitivity may be attacked for the first time.

ANTIBODY PRODUCTION AND GAMMA GLOBULIN IN HUMAN MALARIA

For the first time, the course of antibody production has been followed in human malaria. Although there has been no practical serological test for the detection of antibody produced in human malaria infections, it has been possible not only to detect but to titrate antibody by the fluorescent antibody technique and to correlate this with the appearance of the parasites in the peripheral circulation. In 4 sporozoite-induced Plasmodium vivax infections in human volunteers antibody was detectable several days after the parasites appeared. In a typical case, the antibody rapidly rose to a serum dilution level of 1:2,560,

was maintained at this level for about 40 days after which it gradually fell and remained at a low level for the period of observation (121 days). In 5 patients infected with the B strain of *P. cynomolgi* essentially the same results were obtained and certain of the patients still had detectable antibody about 1 year after infection. The patients showed increased gamma globulin levels above the normal limits. The maximum gamma globulin rise tended to occur at the same time as the maximum antibody titer with a median difference of 3 days. The ability to follow antibody production and increases in gamma globulin levels in controlled malaria infections provides some very basic information necessary in the program connected with the worldwide eradication of malaria.

PROTEOLYTIC ENZYME OF HUMAN SPLEEN PURIFIED

A method has been established for the isolation and purification of a proteolytic enzyme from human spleen which cuts human albumin into large precipitating fragments. By column chromatography it has been shown to be a single type of substance. Characterization of the enzyme as to electrophoretic mobility, pH activity curve and catheptic activity has been determined. Four large fragments of human albumin resulting from the degradation of the native protein by the human enzyme have been detected by immunoelectrophoresis. This study combines several fundamental problems bearing on the nature of antigenicity and the possible roles of enzymes in the degradation of antigens as a primary step in antibody formation.

LUPUS SERUM REACTS WITH MAMMALIAN CHROMOSOMES

Fluorescent antibody studies have demonstrated that when sera from certain patients having lupus erythematosus were applied to mammalian chromosome preparations, these sera reacted as was evidenced by their fluorescence. The sera from the lupus patients had been shown to have anti-nuclear factors. In the case of human chromosomal preparations all 46 chromosomes reacted and became fluorescent. When sera from three patients with Sjögrens syndrome were utilized, the chromosomes did not become fluorescent even though the sera had anti-nuclear factors. It may be possible to find sera or fractions of sera which are more specific in their chromosomal reactions. The anti-nuclear factors found in the sera of patients with the so-called autoimmune diseases are of importance for a better understanding of the mechanisms involved.

GENETIC CONTROL OF GAMMA GLOBULIN ALLOTYPES

In studies on the genetic control of serum allotypes in rabbits, it was previously shown that RGG-I and RGG-II allotypic determinants are under the genetic control of an allelic pair of autosomal genes. Further studies have revealed three other gamma globulin allotypic determinants which are under the genetic control of a three allelic system at a second gene locus. Furthermore, two allotypic determinants were either both present or absent from all sera tested indicating genetic control by a single allele or two closely linked alleles. In addition a gamma globulin at a third locus has been found. The distribution of allotypes varied considerably among several rabbit colonies. The selective breeding of rabbits is in progress to establish genetically defined lines

of rabbits based on the known gamma globulin allotypes. To know the genetic mechanism for the control of allotypes is essential for the usefulness of these allotypes in studies of basic immunology and hypersensitivity.

ALLERGIC THYROIDITIS Delayed hypersensitivity to thyroid extract has been successfully transferred within Strain 13 inbred guinea pigs. Intact lymph node cells from animals actively immunized to thyroid extract were injected into normal recipients. Successful transfer was accomplished only if the lymph node cells were taken early after immunization of the donors. Cells taken 5 days after sensitization successfully transferred skin test hypersensitivity. Lesions of the thyroid were produced by the transfer of lymph node cells in about 30 percent of the recipients. The animal models selected for the study of the autoimmune diseases should aid in the elucidation of the role of circulating antibodies and delayed hypersensitivity in the production of tissue lesions. As more is known about the pathogenesis of these diseases the more intelligent we can be in our approach to the therapy of the autoimmune diseases.

LABORATORY OF BACTERIAL DISEASES

The research program in the Laboratory of Bacterial Diseases has continued to develop in the same general areas noted in last year's report. The major emphasis has been on the projects on Intracellular Parasitism and Staphylococcus.

IN VITRO CELL ANTIBODY In the course of the project on Intracellular Parasitism a study was undertaken of the production of specific antibody by mammalian cells in vitro. This study has been reasonably rewarding and offers considerable promise. Accordingly, at present, the major effort on this project is devoted to this line of investigation.

Specifically, cells are obtained from the peritoneal cavity of laboratory animals, usually the guinea pig. A method has been developed which yields a high proportion of mononuclear cells or macrophages. Further separation of the cell types results in explants which are composed almost entirely of this type of cell. Cells removed from immune animals and properly handled and cultured elaborate specific antibody in vitro for days or even several weeks. The explants result in growth of sheets of cells which are derived from the mononuclear cells or macrophages. On glass they become stellate cells but retain some of the characteristics of macrophages, Such tissue cultures can be maintained for long periods and be carried through at least a number of subcultures. The antigen usually has been egg albumen; the test procedure, hemagglutination of specifically sensitized red blood cells. While production of antibody in vitro has been sporadic, significant titers have been obtained, and in a number of experiments antibody has been detected for periods of several weeks. In comparison with initial cell homogenates and with supernates from cells maintained under non-optimum conditions, the amount of antibody produced exceeds that which can be accounted for by release of preformed antibody and certainly represents new protein synthesis after removal of the cells from the animal.

There is still a question as to the type of cell which is producing antibody and also whether these particular cells have undergone multiplication even though general growth of the tissue culture has occurred. This system allows further study of the factors involved in continued antibody production.

STAPHYLOCOCCUS VIRULENCE In the project on Staphylococcus there is continued emphasis on the factors responsible for virulence of the organism and on basic studies of methods of identification of pathogenic strains of the organism. Gel-diffusion immune precipitation techniques have been applied to standard reference strains of Staphylococcus. Employing methods which tend to eliminate minor and non-specific cross reactions the antigenic mosaic of such strains has been characterized. This technique seems promising for the development of a system of identification. If reference spectra consisting of precipitation patterns formed with specific reference antigens and antisera can be established for staphylococci, this would form a solid basis for the identification of strains.

BRUCELLOSIS DIAGNOSIS We continue to collaborate with other Brucella research centers throughout the world in the development and standardization of laboratory tests for species identification, and for diagnosis of Brucella infection. Brucellosis is still a disease of world wide importance in relation to the welfare of mankind. Control of the disease in domestic animals rests in part upon regional epidemiological studies which require precise identification and classification of strains. A unified effort is currently under way in an attempt to clarify Brucella taxonomy. This undertaking is under the aegis of the Subcommittee on Taxonomy of the Genus Brucella of the International Committee on Bacteriological nomenclature, of which the Chief, Laboratory of Bacterial Diseases, is a member.

LABORATORY OF BIOLOGY OF VIRUSES

Two major changes in the Laboratory of Biology of Viruses during 1961 have resulted in an expansion of its activities. Between July and September, all units of our original laboratory were moved from their scattered locations in Building 7 to be together on one floor in Building 5. The second change was the transfer as a section to the Laboratory of Biology of Viruses of the remainder of the Laboratory of Cell Biology after the retirement of Dr. Harry Eagle.

VIRUS-HOST RELATIONSHIP Investigations of relationships between the virus and its host cell have continued to be the greatest effort of the laboratory. Four different units are working in this important general area, attempting to define the biochemical events, their sequence, site and inter-relationships. These studies represent both basic and practically oriented research. The basic and more direct goal is to determine the mechanism of protein and nucleic acid syntheses in the normal cell, the virus being used only as a self-replicating identifiable biochemical macromolecule. The other more remote goal is the development of knowledge concerning virus invasion and multiplication which might give a

lead as to a logical point of attack in chemotherapy of virus diseases. The past year's work has been productive in this area. Studies on poliovirus in HeLa cells have shown that viral protein and RNA syntheses are closely coordinated in time and increase only a short time before whole infectious virus can be demonstrated. By the use of inhibitors there is evidence that the synthesis of viral RNA may be at least partially dependent upon protein synthesis. Viral protein antigens appear in both the nucleus and cytoplasm, but evidence is lacking as to whether this is the result of synthesis in both areas or migration after synthesis. Studies on vaccinia virus--a DNA virus--suggest that the synthesis of viral DNA and viral protein are dissociated. Work with Coxsackie virus--a RNA virus--shows precursor viral RNA present in the nucleus early after infection, declining by 3 hours and again increasing at 6 hours. Viral RNA in the cytoplasm decreases until 2 hours, then rises continuously to eventual high levels.

VIRUS STRUCTURE

Three units have carried out studies on chemical and physical structure of virus as such. The protein coat of poliovirus has been isolated and found to contain no free N-terminal amino acid residues. The subunit of this protein appears to have a higher molecular weight than the predicted 20,000. A base analogue--5 fluorouracil--has been successfully incorporated into the RNA of poliovirus to the extent of replacing 30% of the normal uracil, but with retention of biological properties of the virus. This "abnormal" virus continues to have all the properties of the parent normal virus as far as infectivity and host range susceptibility. Coxsackie virus has been shown by electron micrographs of purified virus to have an outer envelope and a dense inner core.

VIRUS INHIBITORS

An important practical contribution to virus research technology has been made by the group studying virus inhibitors. The previously demonstrated inhibitor of plaque formation by certain genotypes of certain viruses has been identified as a sulfated polygalactone. Two different practical methods for its removal from agar have been developed as well as materials found which can neutralize the inhibitor. This has made it possible to obtain plaques with strains of ECHO viruses, myxoviruses, Arbor viruses, adenoviruses and rabies virus, all of which produced no plaques by standard methods.

"FOREIGN" CELL ANTIGEN

A study of the mechanism of oncogenesis by polyoma virus has developed evidence that when the virus transforms normal cells to tumor cells it changes the genome of the cell in such a way that a new "foreign" cell antigen appears. This "foreign" antigen is rejected by the immunologically competent adult animal and tumor development is suppressed, whereas the suckling animal being immunologically tolerant cannot reject the new antigen and tumors develop. This demonstrates two important phenomena: 1) the interaction of a virus with a mammalian cell can induce a genetically stable change in the antigenic components of the cell, and 2) immunological competence may be the limiting factor in the progressive development of a tumor after transformation has occurred.

MELANIN GRANULES AND MITOCHONDRIA Important contributions have been made in the general area of normal cell metabolism unrelated to virus infection. In the application of the finding made last year that tetracycline antibiotics localized specifically on mitochondria and can be demonstrated by fluorescent microscopy, it has been found that melanin granules in melanoma tumors act like mitochondria, thus adding another bit of evidence for the mitochondrial nature of these granules.

The development of a mutant line of HeLa cells resistant to the toxic effects of a glucose analogue has led to the demonstration that this drug resistance is due to the presence of an inhibitor of hexose phosphorylation. This inhibitor may be related to alkaline phosphatase since the latter is 8 to 10-fold higher in the resistant cells. Studies using a DNA inhibitor in HeLa cells have produced evidence that synthesis of protein and RNA in the nuclous is DNA-dependent, whereas their synthesis in the cytoplasm is not. In a continuation of studies on inborn metabolic defects, initiated by another investigator, current studies of cell cultures from gout patients show these cells to produce and release large quantities of purines compared to normal cells.

New areas of investigation initiated late in the year include the genetics of viruses and cells in tissue culture systems and the study of the mechanism of recovery from virus infection with special emphasis on interferon.

LABORATORY OF PARASITE CHEMOTHERAPY

The United States is an active participant in world-wide malaria eradication. Our principal contributions to the effort arise from basic and clinical studies on the disease. In addition, we are committed to the same kind of investigations involving parasitic infections in general, with special emphasis on schistosomiasis and intestinal parasites. Because of the zoonotic character of simian malaria, as demonstrated here in 1960, and a large interest by the senior staff in overall problems of malaria, the main research effort during the year has been in that field.

MALARIA - SIMIAN Man-mosquito-man transmission was accomplished consistently with two different strains (B & M) of Plasmodium cynomolgi, even with low gametocytemias. Man-mosquito-monkey transmission was accomplished with each strain also.

Infections produced by the inoculation of blood parasitized with the B or the M strain of P. cynomolgi exhibited no significant differences in clinical manifestations. However, when these infections were induced in volunteers by mosquito bites, several differences were noted; in the M strain, the duration and height of the fevers were greater, the tertian patterns were more numerous and typical, and splenomegaly more frequent.

FAR EAST RESEARCH UNIT STUDIES IN MALAYA

In Malaya, approximately 33 percent of pig-tailed macaques (*Macaca nemestrina*) and about 40 percent of the long-tailed macaques (*Macaca irus*) are infected with a malaria. A new species of malaria was isolated from this latter species and was designated *Plasmodium fieldi* in honor of Dr. John Field. *M. irus* also harbored *P. knowlesi* and *P. inui*. *Hepatocystis semnopetheci* was discovered for the first time in *M. nemestrina*.

Malaria parasites were also demonstrated in *Macaca irus* and *M. nemestrina leonina* from Thailand and from the latter monkey from East Pakistan. *Plasmodium knowlesi* and *P. inui*, along with a species of *Hepatocystis*, were found in *M. irus lacta* from an isolated island off the east coast of Malaya.

A significant proportion of all three Malayan species of leaf monkeys (*Presbytis cristatus*, *P. obscurus*, and *P. malalophos*) were shown to be infected with malaria. Exact species determination of the parasites have not been made.

Anopheles hackeri was shown to be a natural vector of *P. knowlesi* and at least two, possibly three, tertian-type parasites so far unidentified. *A. maculatus*, *A. sundiacus*, *A. leucosphyrus*, *A. umbrosus* and *A. letifer* were found to bite simians but so far there is no evidence that any transmit malaria. Experimentally, it was shown that *A. maculatus*, *A. sundiacus*, *A. philippinensis*, *A. kochi* and *A. hackeri* are susceptible to infection with *P. cynomolgi*.

MALARIA - HUMAN

Plasmodium falciparum from Colombia, South America was found to be resistant to several members of the important 4-aminoquinoline group of drugs, i.e., chloroquine, amodiaquine, and hydroxychloroquine. Because of the wide-spread use of these drugs, such resistance, if prevalent, could be of major significance. Resistance to mepacrine was present also. Resistance has now been shown, by certain malaria parasites, to each of the important groups of synthetic antimalarial drugs developed in the last 30 years.

A drug (BW-377C54), with reported activity against human malaria similar to that of chloroquine, failed to cure *P. falciparum*. Also, the Colombia strain of *P. falciparum* appears to be resistant to this compound.

A study of the life-pattern of Venezuelan vivax malaria indicates that it has an intermediate-type latent period following treatment of the initial attack. This differs from the short-term (Chesson) or long-term (St. Elizabeth) latent period of other strains.

The chloroquine-resistant strain of *P. falciparum* contains a characteristic component which can be detected in hydrolyzed RNA. This suggests a chemical method for identifying resistant strains of malaria.

BIOCHEMICAL STUDIES

Analysis of ribonucleic acid derived from *Plasmodium gallinaceum*, *P. berghei*, *P. cynomolgi*, *P. inui* and *P. gonderi* revealed the presence of nucleotides of the usual purine and pyrimidine bases: adenine, guanine, cytosine and uracil with adenine most abundant and cytosine the least abundant. In addition, nucleotides of hypoxanthine are found indicating the activity of adenosine deaminase.

Significant binding of chloroquine to a component or components in plasma, but not to pure serum albumin, was demonstrated. The degree of binding of this drug to blood cellular elements in vitro could be altered by a number of physical means. Antimalarial drugs, energy sources, and metabolic inhibitors were studied to determine their effect on the incorporation of amino acids into malaria protein. None of the antimalarial drugs had an effect on this process or on glycolysis.

Nucleic acid extracted from a pyrimethamine-resistant strain of *P. gallinaceum* hastened the development of resistance in a normal strain exposed to both drug and the extract. A decrease in chloroquine sensitivity of *P. berghei* has been observed following exposure of infected donor mice to 1,000 roentgens of X-radiation.

NUTRITION AND MALARIA

Nutritional deficiencies that favor the development of peak parasitemia in mice infected with *Plasmodium berghei* include folic acid, pantothenic acid, and niacin.

Deficiencies suppressing parasitemia include para-aminobenzoic acid and riboflavin. Aminopterin, a folic acid antagonist, also suppressed peak parasitemia. Thiamine deficiency appeared to have no effect. Deficiencies were studied both in the absence and presence of suppressive levels of chloroquine. In only one case did deficiencies have a reverse effect; pyrodoxine deficiencies appeared to favor the parasite in the presence of the drug but suppressed the parasites in the absence of the drug.

INTESTINAL PARASITES

Bephenium chloride, given for three or five days, was highly effective against hookworm infections and also removed 68 and 88 percent, respectively, of *Trichuris trichiura* worms. A rural population heavily infected with *Ascaris* lost 90 percent of their worms when given single doses of piperazine. Hookworm and *T. trichiura* infections were shown to persist for at least seven years in institutionalized mental patients.

Tapeworms (*Hymenolepis diminuta*) exposed to mepacrine (Atabrine) *in vivo* were resistant to the action of the drug *in vitro*. This is interesting to follow up because it suggests a type of resistance not based on selection but upon physiologic adaptation of individual organisms.

Evidence suggests that three anthelmintics, bephenium, mepacrine, and dichlorophen, are effective by inhibiting the enzymes located on the outer surface of tapeworms.

INSECT TISSUE CULTURES Lepidopteran tissues have undergone growth or extended maintenance in culture. In cultures of caterpillar hemocytes, SLE virus has been maintained up to 10 days.

Prepupae and pupae fat body cells readily proliferate in hanging drop culture for 10-14 days; SLE virus persists in such cultures and may increase between the 6th and 8th day.

VIRUS-MOSQUITO LARVAE ASSOCIATIONS Larvae of *Aedes aegypti* and *Culex pipiens* mosquitoes acquired SLE viruses from an experimentally contaminated aquatic environment. The virus persisted through the subsequent pupae stage and into the adult insect. There have been nine confirmed transmissions of the virus by bites of the *Aedes* and nineteen by the *Culex* mosquitoes.

VIRUS-PARASITE COMBINATIONS *Plasmodium gallinaceum* reduced the quantity of circulating encephalomyocarditis virus in chicks. Additional experiments have continued to verify the role of *P. berghei* in the transport of SLE virus to the central nervous system in mice when the virus is given intraperitoneally.

LABORATORY OF PARASITIC DISEASES

AXENIC CULTURE OF ENTAMOEBA HISTOLYTICA Work on the cultivation of *Entamoeba histolytica* in the absence of any other living organisms has progressed steadily. Most important is the demonstration that the technique of developing such axenic cultures is applicable to more than one strain. A strain of the amoeba very recently isolated from a human patient has been established in axenic culture. Secondly, the culture medium has been further simplified, with the elimination of chick embryo extract. Also, it has been possible to eliminate agar from the liquid overlay, a technical factor of importance in that it will eventually aid in evaluating culture growth.

FREEZING PROTOZOA Freezing of protozoa in liquid nitrogen has been developed to the point of practicability. *Entamoeba histolytica* has been kept viable for at least 10 months and *Trichomonas vaginalis* for six months; there have been no losses in viability by the use of the technique. Other tests underway clearly demonstrate that *Entamoeba invadens*, *Trichomonas hominis*, *T. gallinae*, and *T. foetus* can be maintained viable in liquid nitrogen. These experiences indicate that indefinite storage of protozoal strains is feasible.

FILARIID LARVAE IN VITRO The development of techniques for freeing microfilariae completely from blood cells and the demonstration of development of microfilariae to the sausage stage are significant advances. This is the first time that sausage stages have been found with any regularity in *in vitro* preparations. Additional work can now be expected to result in further development of filariid larvae *in vitro*. Survival of adult *Dirofilaria uniformis* in chemically defined tissue culture nutrients (NCTC 109 + 10% serum) has been obtained for as long as 35 days.

Microfilariae were discharged from the females during the first 2 to 3 weeks, only when serum was present in the medium. Under these conditions for maintenance of the filariids, glucose was transformed quantitatively to lactate.

NEMATODE CYCLE IN GERMFREE ANIMALS

In the study of the development of Nematospiroides dubius in germfree animals, the nematode has now been carried through its entire life cycle three times in the absence of bacteria. Larvae from the feces of germfree mice have been cultivated axenically to the infective stage and then used to reinfect new germfree mice. It is noteworthy that infections thus produced in germfree animals are not as extensive as in conventional animals; worm burden, worm size, and egg-production appear reduced. This may represent an important lead in determining the factors responsible for alterations in host-parasite balance. The possibility of a steroid action, which is in some way dependent upon the microbial activity in the intestinal tract, is suggested by the differences between conventional and germfree animals in respect to the worm recovery in male versus female mice.

NUTRITION AND SCHISTOSOMIASIS

The study on the relation of nutrition to schistosomiasis in Puerto Rico is almost complete. Liver function tests returned to normal in all schistosome-infected patients who were placed on the high protein, high caloric diet. Stibophen (Fuadin) levels were higher and maintained longer in the same patients.

Studies on the mechanisms which produce higher blood levels of stibophen in human beings on high protein high calorie diet have been accomplished in mice on complete semi-synthetic diets in which enhanced activity of stibophen is observed. The higher levels of drug are attributable to the acid-base relations of salts in the diet. With acidic salts Fuadin activity is enhanced. Similarly the efficacy of antimony potassium tartrate, antimony (III) sodium meso-2 3-dimercaptosuccinate (Astiban) and pararosaniline pamoate for killing mature Schistosoma mansoni in mice has been found to be increased up to 16X in mice maintained on the semi-synthetic diet compared to mice on the commercial pellet diet.

BIOCHEMICAL STUDIES

Investigations on Trypanosoma cruzi have revealed the fixation of carbon dioxide by this organism, with the incorporation of the CO_2 into succinic acid. Degradation of succinic and acetic acids from incubates containing C^{14} labeled glucose has established that most of the carbons of these acids are derived from glucose. Preliminary fractionation of the phospholipids of T. cruzi has yielded serine and ethanolamine as well as an unidentified ninhydrin-positive fraction. CO_2-fixation is known to occur in a number of free-living and parasitic protozoa, and some parasitic helminths. It appears that CO_2 functions as a metabolite as well as appearing as an excretory product.

The studies on the loss of sugars from Cysticercus fasciolaris and Taenia taeniaeformis in sugar-free solutions and the absorption of glucose and galactose when these are available are basic observations in work that is being continued on the kinetics of absorption of nutrients through the cestode surface. It can be expected that further work in this direction may lead to demonstration of active and selective transport. In connection

with the cestode studies it is of interest to note that the data show the metabolism of larval and adult worms to be proportional to fractional powers of the body weight intermediate between those characteristic for animals with increase in metabolic rate directly related to weight and for animals with increase in metabolism proportial to surface area. This was an unexpected finding because in tapeworms surface and body weight increase in the same ratio, and hence an increase in metabolic rate paralleling weight increase had been anticipated. Also, the quantitative, rather than qualitative, nature of differences in metabolism of adult and larval worms is noteworthy, since in other forms of parasites, e.g. trypanosomes, there are qualitive differences between various forms.

Additional work on the calcareous corpuscles of *T. taeniaeformis* in comparison with artifical mixtures of appropriate salts indicates that the dolomite structure must be preformed in some way in the corpuscles, although they are definitely amorphous until heated. This is of considerable biological and geological interest.

Biochemical studies on intact rat liver mitochondria have shown that a reversal of DPN-flavin-linked oxidative phosphorylation is involved in the reduction of acetoacetate to succinate, with an energy transfer equivalent to one high-energy bond per molecule of acetoacetate reduced. This energy can be supplied by one or both of the two terminal respiratory chain phosphorylations without the intermediary of extramitochondrial ATP. Oxaloacetic acid inhibits oxidation of succinate by DPN-depleted mitochondria. The inhibition is not of the classical competitive type but can be overcome by added ATP. Mitochondria inhibited by oxaloacetate gradually regain ability to oxidize succinate after the oxaloacetate is removed. These findings support concepts previously advanced that there is a compartmentation of substrate-level phosphorylation within mitochondria. This work, on basic biochemical pathways, is of general importance and obviously may be useful in explaining mechanisms for succinate metabolism in parasites.

VIRUS POTENTIATION BY TRICHINA

The potentiation of encephalomyocarditis virus by *Trichinella spiralis* in mice is closely related to the size of the worm inoculum and the time in the worm infection that the virus challenge is given. A similar phenomenon, of enhanced multiplication of Coxsackie A-9 virus in murine muscle, is again related to *T. spiralis* infection. The studies on *Taenia taeniaeformis* infection in mice, which seem to equate natural resistance with an accelerated response of acquired resistance, are of general interest. The demonstration of a possible immune tolerance in animals receiving *T. taeniaeformis* antigen shortly after birth, is of considerable significance; this phenomenon has not been demonstrated previously in relation to parasites.

EXPERIMENTAL SCHISTOSOMIASIS

Continued work on the pathology of schistosomiasis has revealed differences in response among mice, hamsters, and multimammate rats (*Mastomys*) as to the vascular lesions seen in the liver. Mice and *Mastomys* develop more lesions than hamsters. The explanation for this may be that the lesions are at least partly due to allergic reactions and the hamster is notably less reactive in this respect. The development of hepatosplenic schistosomiasis occurs

in mice even with very small worm burdens; a longer period of time is required for the syndrome to appear. The cor pulmonale picture produced in schistosome-infected mice by partial portal vein ligation provides another experimental model for studying clinical schistosomiasis.

TOXOPLASMOSIS Toxoplasma gondii has been found in the ovaries and oviducts of healthy chickens. The organism is present in the cyst form that can survive digestion. The possibility that it can be found in shelled eggs is important in regard to the epidemiology of toxoplasmosis. Also, it has a technical importance relative to the use of chick embryos for cultivation of viruses and rickettsiae.

In sheep, resistance to congenital transmission of toxoplasmosis is manifest only against moderate challenges. No protection is manifested against high challenge doses. This is of interest in human medicine. Exposure to large numbers of Toxoplasma can occur in women of particular population groups that customarily enjoy raw meat, and under such circumstances it is possible that habitual abortion may occur. This has been reported from Germany but not from the United States.

Electron microscopy of Toxoplasma has revealed a schizogonic reproductive process, sometimes with the production of two filial parasites within a parent cell and sometimes apparently with the production of small rosettes. A third process, in which two organisms are involved, may represent longitudinal fission or a sexual fusion. These new observations lend support to the concept that Toxoplasma belongs among the Sporozoa of the Protozoa, probably in a new sub-class along with organisms such as Besnoitia and Sarcocystis.

Under the electron microscope, the formation and character of the cyst wall of Toxoplasma has been studied. The wall arises from interaction of the parasite and the host cell. The nature and development of the cyst are important in understanding chronic infection. Although some activity of cysts is evident in chronic infection, long-term treatment with pyrimethamine begun as early as three weeks after infection can produce some diminution in residual brain infection but cannot eliminate it.

Progress has been made in the fractionation of Toxoplasma antigens. Separation of hemagglutinating antigen on a hydroxyl apatite column yields an initial fraction that induces hemagglutinating antibodies but not dye test antibodies when injected into rabbits. This fraction has spectrophotometric activity indicating a high proportion of nucleoprotein, presumably from the cytoplasm of the parasite. Attempts to get a "clean" cell wall preparation to induce dye test antibody formation in the absence of HA antibody have thus far been only partially successful, in that such fractions have stimulated dye test antibodies more rapidly. If the complete separation can be attained, one can conclude that dye test antibodies are produced against the cell wall and that hemagglutinating and complement fixing antibodies are stimulated by the intracellular components of the parasite.

LABORATORY OF TROPICAL VIROLOGY

The third year of the existence of the Laboratory has seen continuation of field-laboratory investigation on viral disease at the Middle America Research Unit in the Panama Canal Zone and supportive laboratory studies in Bethesda.

VENEZUELAN EQUINE ENCEPHALITIS (VEE) The first isolation of this virus in Panama was accomplished in April from specimens obtained from a dying Panamanian boy. Three senior laboratory staff members became infected as a result of the exposure (with virus isolation and serological confirmation in each case). The entire MARU contingent was then immunized with live attenuated VEE virus vaccine. Mild reactions were often encountered, and the attenuated virus was isolated from some of the vaccinees. Several aspects of practical application of this still experimental preparation were thus explored.

Epidemiologic and ecologic studies of the rural community where the case had occurred (in collaboration with Gorgas Memorial Laboratory), failed to demonstrate the VEE virus in wild, domestic or sentinel animals, or the mosquitoes collected. Serological evidence of VEE infection (CF, HI, NT) was established for a significant percentage of residents of the area.

A remarkable urban epidemic of VEE occurred in another part of Panama near the site of the Gorgas Memorial Laboratory field station. Approximately 350 cases were registered during a two months' period. Important new information on the epidemiology and the clinical spectrum of epidemic infection with this virus is being developed by GML and MARU.

VESICULAR STOMATITIS VIRUS (VSV) Last year we reported repeated isolation of Indiana type VSV from phlebotomus sandflies collected in the course of a collaborative project with Gorgas Memorial Laboratory on the ecology of arthropod-borne viruses. Approximately 700 human sera were collected from the population of a town near the field station. One-fourth of the 490 sera tested had neutralizing antibodies to Indiana type VSV. One-fourth of the neutralizing sera also possessed CF antibodies.

Interest in this virus prompted investigation of an outbreak of vesicular stomatitis in cattle. The causative agent was found to be the New Jersey, rather than Indiana, type of VS virus. Antibody patterns of humans in close or remote association with the infected cattle suggested that direct contact, rather than arthropod vectors, may be involved in the transmission of this infection to man. It would seem that the ecology and epidemiology of the two types of VSV are quite dissimilar.

VIRUS FROM RAIN FOREST ARTHROPODS During the second year of the study on the ecology of arthropod-borne viruses in the tropical rain forest of Panama, conducted by GML (NIH grant E-2984) in collaboration with MARU, the number of virus isolates was doubled: present total 38 - 14 from mosquitoes and 10 from phlebotomus sandflies.

The isolation rate from sandflies continues to exceed by far the overall isolation rate from mosquitoes.

The first isolate of Indiana VSV from a *non*-sandfly source was recorded (mosquito *Culex nigripalpus*). Many of our mosquito isolates have been identified with collaboration of the Trinidad and Belem laboratories of the Rockefeller Foundation: six strains closely related to *Una* virus of Group A (work with four other similar agents has not been completed); one strain of Ilheus virus of Group B; several members of the *Bunyamwera* group-one *Guaroa* and four *Wyeomyia* viruses; *Guama* group, represented by six isolates, all from the same mosquito (*Culex vomerifer*). A few other agents remain unidentified.

ATTEMPTS TO RECOVER VIRUSES FROM PARASITIC MITES Nearly 500 pools of acarines (including 25 species of chiggers, 35 of parasitoid mites and a few ticks from over 350 wild vertebrates) were inoculated into suckling mice and hamster kidney cell cultures. Not a single viral agent was isolated, attesting to the uncommon presence of at least the recognizable viruses among the common tropical mites. Many ectoparasite pools were shipped to the Rocky Mountain Laboratory for attempted recovery of rickettsia. One positive isolation of the *Coxiella burnetii*-like agent was made from a species of intradermal chigger, parasitic on the tropical spiny rat.

MYCOSES Repeated isolation of *Histoplasma capsulatum* from bat liver and spleen suspensions was a most important finding, providing further evidence for the suspected role of bats in the ecology of histoplasmosis. The first human case of actinomycosis (cervical-facial type) caused by *Actinomyces bovis* was recorded demonstrating the existence of this disease on the Isthmus of Panama.

ROCKY MOUNTAIN LABORATORY

Investigations have continued with the same general objectives as previously; those of one large group are oriented toward the zoonoses and arthropod-borne diseases, and those of another major group are chiefly concerned with problems related to allergy, immunology, and resistance to disease. Both divisions involve basic laboratory research some of which could lead ultimately to development of applied and control measures. While investigations of various vector-borne illnesses have never actually diminished, occasional changes in emphasis have occurred in the last few years with new leads, such as provided in encephalitis ecology by the mosquito-garter-snake relationship, discovery of new foci of Powassan and California viruses, and variations in spotted-fever-like isolates. On the other hand, attacks on phases of such nonvector-borne disease problems as tuberculosis, *Salmonella*, pertussis, poliomyelitis, influenza, and certain mycoses have been intensified in varying degrees.

TICK PARALYSIS Inbreeding through 4 generations of a paralysis-producing strain of ticks from British Columbia has not produced an increase in the ability to cause paralysis, probably because of our inability to select for the genetic factor in male ticks. However, a new lead on genetic aspects may be the sudden acquisition of paralysis-producing ability by some females resulting from a cross of "paralytic" males with females from our previously "nonparalytic" laboratory stock.

TULAREMIA While it was reported under *Pasteurella tularensis* last year that "the protection afforded by live organisms was not effective for long periods of time," mice later vaccinated with a living Russian attenuated strain had a high level of immunity for more than 30 weeks.

Further comparison of isolates from different sources and geographical localities has strengthened the belief that a "fully virulent" organism in North America causes severe disease in sheep, hares, rabbits, horses, and man, whereas a less virulent form is common to North America, Europe, and Asia and may require systematic differentiation.

DISEASES OF WILDLIFE *Corynebacterium pyogenes* was cultured from 3 dead mountain sheep during an epizootic and from 1 deer and 1 elk. These findings suggest that this may be a pathogen of consequence to certain wildlife, as well as to domestic animals.

Three more isolations of rabies from bats this year bring the local total to 24. Contrary to most concepts, rabies can be chronic in mice. A consequent discovery was that recovered mice with limb movement impaired by rabies became heavily infested with ectoparasites. Mechanical interference with combing and grooming, caused by amputating limbs of normal mice, resulted in similar excessive parasite populations. Another unidentified, nonfatal virus was incidentally isolated from bats and passed in tissue cultures and mice.

In the absence of a usable skin test for detecting the protozoon, *Toxoplasma microti* in *Microtus*, an effective method that does not cause permanent injury to the animal was devised for observing brain lesions through a slit in the scalps of mice. Tolerance for this organism may result from *in utero*, or very early postnatal, infection in native mice.

A fungus, *Emmonsia crescens*, found in certain indigenous small animals of several continents, may also infect man, as indicated by positive passive cutaneous anaphylaxis tests applied to human serums.

ARTHROPOD-BORNE VIRUSES Western equine encephalitis virus from *Culex tarsalis* at Vale, Oregon, was isolated during the midsummers of 1960 and 1961, but not in the intervening months from October to May or from bird tissues collected during this period. These findings suggest that latent infection in birds during the summer months is unlikely. The overwintering virus mechanism still remains illusive, though recovery of virus in the spring of 1961 from captive garter snakes that had been bitten before hibernation by infected mosquitoes in the fall of 1960 is

suggestive, in spite of failure, as yet, to show natural infection in such snakes. This lead has been a popular one and has caused examination of reptile-virus relationships in many countries.

Three isolates of California virus have been made in the Bitterroot Valley from ticks off a snowshoe hare, a golden-mantled ground squirrel, and a chipmunk. Antibodies occurred in a low percentage of these local animals. This naturally leads to an inquiry into the role of ticks as vectors.

An additional isolation of Powassan virus from ticks in western South Dakota has resulted in intensified ecological studies in this area with consequent finding of antibodies in serums from local chipmunks and wild mice. A human serum from western Minnesota and one from central North Dakota (of 1,058 miscellaneous samples tested) showed high neutralizing indexes against this virus. Regional field work will be focused on ecologic and public health aspects of this virus, in view of the known significance of the related Russian spring-summer encephalitis.

Q FEVER Field investigations this year were oriented toward a more objective evaluation of the potential public health problem associated with the vast reservoir of infection among dairy cattle. As determined through continuous surveillance, even minor illness among persons exposed to infected dairy cattle could not be attributed to Q fever. However, 87% of persons exposed to infected herds have acquired antibodies detectable by the mouse-neutralization test and the radioisotope precipitation test.

Investigations on developing a safe method of vaccinating humans with conventional Q fever vaccine were continued at Montana State Prison. Most of the skin-test negative volunteers (97%) who last year received from 1 to 3 doses of 10 complement-fixing (CF) units of antigen subcutaneously reacted positively on a skin test given 10 months after vaccination. However, the agglutinin response among groups of vaccinees varied from 45 to 91% and was directly proportional to the number of doses of vaccine received. Studies on a comparable group of volunteers who received from 1 to 3 doses of 1 CF unit of antigen intradermally are not complete, but results to date are similar to those obtained when vaccine was given subcutaneously. Although the resistance induced in human volunteers could not be challenged, vaccination appears to have been effective, as judged by available immunologic and serologic data.

Significant progress has been made in investigations directed toward fractionating _C_. _burnetii_ into its component antigens. The separation of nontoxic immunogenic fractions from those responsible for inducing or eliciting the hypersensitive state remains a major objective. All antigens of any consequence were determined to be located in the cell wall. Chemical extraction of whole phase I rickettsias with dimethyl acetamide, dimethyl sulfoxide, or a combination of trichloroacetic acid and phenol have yielded protective antigens whose toxicity and ability to induce an allergic response were decreased about 100-fold. Similar extracts of phase II organisms were nonimmunogenic and, in preliminary comparisons of whole phase I and II rickettsias, phase I organisms were more potent vaccines.

24

ROCKY MOUNTAIN SPOTTED FEVER

Because of the results of recent studies on rickettsial variants and observations made possible by fluorescent microscopy, interest in the biology of spotted fever rickettsias has been rekindled. Particular attention has been given to the elucidation of factors which affect the natural infection rate among ticks and to the characterization of spotted fever antigen in naturally infected ticks. Of ticks collected in nature, up to 25% contain specific antigen detectable by fluorescent microscopy but only 1 to 3% contain pathogenic rickettsias.

The ecology of *R. rickettsii* appeared to be the same in 2 local areas which had similar natural features. Heaviest infestations of immature *Dermacentor andersoni* occurred on golden-mantled ground squirrels and chipmunks, and virulent organisms were isolated from these rodents for the first time west of the Mississippi.

Heretofore, 4 variant types of *R. rickettsii* in *D. andersoni* have been recognized. The spectrum of pathogenicity of these types varies from an ability to cause overt disease and death to a limited ability to cause only immunizing, inapparent infections. From ticks collected in eastern Montana, many isolates of a fifth type have been recovered. These strains are nonpathogenic for guinea pigs and mice and appear to be antigenically different from other types of *R. rickettsii*. The relationship of the fifth type to the ecology of spotted fever rickettsia remains to be clarified.

By the use of the fluorescent antibody technique, the gross discrepancy between the incidence of pathogenic rickettsias among *D. andersoni* and the prevalence of specific antigen in this tick has been tentatively explained by the demonstration of a rickettsia like organism that is noninfectious for chick embryos as well as for guinea pigs. This agent behaves like *R. rickettsii* because it is transovarially transmitted by *D. andersoni*, has a similar distribution in tick tissues, and is indistinguishable from *R. rickettsii* by fluorescent microscopy. Studies will be continued to determine whether this organism may influence the prevalence of pathogenic rickettsias among ticks in nature.

MECHANISMS OF ALLERGIC PHENOMENA

A particular skin protein which had been altered by combination with a simple chemical plays an important role in contact allergy. When a conjugate of a purified heterologous protein and hapten was used, antibody but not contact hypersensitivity to the hapten developed. However, when a conjugate of a simple chemical and a soluble fraction from guinea pig skin was used as a sensitizing agent, contact hypersensitivity to the protein developed. This clearly shows that contact hypersensitivity, such as poison ivy rash, is dependent upon combination of chemicals with substances in the skin.

Since the delayed hypersensitive state can not be quantitated by conventional tests in the skin of sensitized animals, an attempt was made to use the cornea as a site for measuring response to the antigen because this tissue is not vascularized. In animals sensitized with a conjugate of protein and hapten, the reactions in the cornea occurred only to the protein moiety,

although circulating antibody to the hapten and associated allergic reactions could be demonstrated in other tissues. In the corneal response, which is primarily polymorphonuclear, leucocytes from surrounding tissue and blood vessels were chemotactically attracted to the antigen.

Although delayed shock is thought to be a systemic manifestation of delayed hypersensitivity, the reaction apparently is dependent also upon circulating antibody. In guinea pigs sensitized with hapten-protein conjugates, only injection of the protein causes the systemic delayed reaction when only delayed hypersensitivity to the conjugate is present. However, after antibodies have appeared, intraperitoneal injection of the conjugate causes a brief initial hyperthermia followed by a distinct hypothermia and lymphopenia typical of delayed shock. At this time delayed shock can not be precipitated by the injection of protein alone but it can be produced by injection of hapten combined with a heterologous protein.

Although many immunologic reactions seen in comparative studies of allergic phenomena in newborn and adult guinea pigs were similar, the delayed response evoked by intradermal injection of antigen could not be elicited before 14 days after birth. This unresponsiveness is attributed to the inability to excite a normal inflammatory response, but the basis for this inertia has not been fully clarified.

To date, these and related studies on tolerance and the effect of hormones and vitamins on delayed allergy support the thesis that delayed hypersensitivity and antibody formation are but phases of the same process. Fundamental knowledge of the mechanisms involved should provide some insight into the study of allergic phenomena associated with infectious and autoimmune diseases.

ENDOTOXINS Although the exact chemical composition of endotoxin has not been defined, the biological activity of endotoxin complexes, contrary to previous conceptions, cannot be associated with the lipid fractions, and the active principle is probably a polysaccharide.

In further studies, in which endotoxin complexes were depolymerized by treatment with dilute acid, biological activity, including pyrogenicity, tumor damage, lethality, and immunogenicity, was associated with the size of the molecule. When depolymerization had progressed to the extent that the haptenic units were about 1/100th the size of the original endotoxins, all biological activity was eliminated. These studies indicate that one of the major requirements for endotoxin to elicit host reactions is a macromolecular complex of critical size.

Other methods of depolymerizing and recombining the haptenic units will be used to clarify the relationship between molecular size and biologic activity.

MORPHOLOGICAL ELEMENTS OF MICROORGANISMS Through collaboration with designers and engineers of a firm specializing in the manufacture of laboratory instruments, a self-contained refrigerated cell-fractionator patterned after the prototype made at RML was tested and developed for the market. The machine is designed so that biological materials can be

subjected to any selected pressure up to 60,000 psi and then by sudden release of material through a cooled orifice, organisms or tissue cells can be disintegrated without denaturation of any of the cellular components. During the past year, 12 guest workers and visitors came to RML specifically for the purpose of using this instrument or learning more about it. Since cellular material, varying from the size of small bacteria to large mammalian cells, can be completely disintegrated without denaturing any of the chemical constituents, this instrument will have varied and extensive application in diverse problems in bio-medical research.

RADIOISOTOPE PRECIPITATION The development and application of a radioisotope precipitation technique (RIP test) to serology of virus diseases and the study of virus synthesis received major consideration. This technique is based on the principle that radioactive particulate antigen (25 mμ to 300 mμ in diameter) sensitized by specific antibody is agglutinated after species-homologous antiglobulin is added to the reaction. No removal of excess globulin is necessary as it is in conventional Coombs type tests where visible precipitate, rather than removal of radioactivity from suspension, is the indicator. The test has been standardized so that it possesses a high degree of sensitivity and specificity. In the determination of specific antibody, the test is 30-fold more sensitive than the tissue-culture neutralization test for poliovirus and 100-fold more sensitive than agglutination or complement-fixation tests for Q fever. By the use of an inhibition-type modification, it is possible to detect as little as 0.1 to 0.001 μg of antigen. The inhibition-type modification has been found particularly useful in studies of viral metabolism wherein it is necessary to detect specific viruses, viral components, and enzymes which may be stimulated by RNA and DNA of plant and animal viruses.

LABORATORY OF GERMFREE ANIMAL RESEARCH

Interest in the use of germfree animals in an increasing variety of research projects continues to grow. More and more, members of our staff are asked to lecture on germfree animal research, techniques, and applications, at scientific sessions and before university staff. At present, in addition to collaborative work with the staff of other laboratories in our own Institute, we are engaged in projects with scientists of the Cancer Institute, CDC, and three universities. It is anticipated that this type of cooperation will continue, especially when the cooperating groups have materials, techniques, and tests which will be helpful to us, but which would not be worthwhile for us to attempt to set up.

The possibilities offered by germfree, virus-free, or virus-defined animals for serologic, tissue culture and perhaps pharmacologic standards

will, it is felt, receive increasingly more attention, and has been the subject of discussion among scientists working with germfree animals.

SUSCEPTIBILITY BY SEX

In the collaborative studies with the Laboratory of Parasitic Diseases the early impression that the sex effect known to occur in certain helminth infections in conventional animals does not occur in germfree animals has been corroborated several times. Whatever the mechanism involved, the lack of a flora reverses the effect normally shown in conventional females. The latter are poor hosts for *Nematospiroides dubius*, for example. We are currently studying mono-infections with individual species of intestinal bacteria in an effort to see if we can pinpoint this interesting relationship. Studies by others working with mono-infected animals have shown that individual species of bacteria can bring about a specific physiologic effect. Among these relationships has been a demonstration of the effect of bacteria on steroid compounds *in vivo*.

DIETARY EFFECT ON HELMINTH INFECTIONS

An effect of diet on the course, and duration, of certain helminth infections has been observed. This effect appears to be holding up in germfree animals as well as in those reared under conventional circumstances. These infections do not do as well in an animal on a semi-synthetic diet of casein, carbohydrate, vitamins, etc., as they do on the stock Purina-type laboratory animal feed. However, the germfree state appears to compound the difference.

"NATURAL" ANTIBODIES

In studies on the occurrence of so-called "natural" antibodies in uninoculated animals, both germfree and conventional, several interesting findings have been made. We have found that mice from a colony which has been reared for at least 7 years free from viable *E. coli*, *S. typhosa*, etc., show about the same reactivity to Gram-negative organisms and their products as do conventional animals. No differences were obtained in levels of bactericidal antibody, resistance to endotoxin and phagocytic response. This is of particular interest inasmuch as it had been postulated by others (in theoretical discussions of modes of action of endotoxin) that germfree animals might be considerably more resistant to endotoxin because of their lack of exposure to Gram-negative organisms. It would appear that even though there are no viable, metabolizing, Gram-negative organisms in the animal's intestinal tract, repeated ingestion of small doses of the heat-stabile endotoxin material inevitably present in the diet is sufficient to stimulate the animals.

A similar situation seems to prevail with respect to antibodies or antibody-like activities against *Staphylococcus* antigen. In a variety of tests involving serum-gel-diffusion, agglutination, and fluorescent antibody techniques, uninoculated animals not harboring any bacteria that could

be detected still demonstrated staph antibodies. It was noted that the germfree animals did not develop these "natural" antibodies at as early an age as did the conventional animals. Animals 2-3 months of age were negative, whereas those 7-8 months of age showed considerable activity. Again, one must conclude that prolonged ingestion of small amounts of heat-stabile staphylococcal antigenic material (it has recently been demonstrated that such substances exist) can stimulate the formation of antibodies. These findings point to the source of some of the "natural" antibodies that occur in uninoculated animals. Eventually, we may have to resort to animals. raised on soluble diets of small-molecule materials prepared under conditions which (hopefully) would preclude the presence of these, apparently, ubiquitous heat-stabile antigenic materials.

MOUSE COLONY FREE OF VIRUS

In our continuing search for evidence of the presence of viruses in our mouse colony, the results continue to be essentially negative for most of the viruses tested. In samplings of well over 125 animals there has been the suspicion of the presence of only one virus in a few instances-- Reo 3. Attempts to isolate the agent from bedding, etc., from colony units have not been successful. Among other viruses tested for which negative results were obtained are included mouse adenovirus, poloyma, GD VII, hepatitis and K virus. These studies will continue until we are reasonably sure of the viral state of our colony. However, thus far, it would appear that our germfree mice are free of some of the more common mouse viruses plaguing viral studies in conventional animals.

THYROIDITIS IN GUINEA PIG

In collaborative studies with the Laboratory of Immunology, thyroiditis has been produced in some guinea pigs as early as 5 days after immunization. This constitutes the earliest recorded observations of this autoimmune disease in experimental animals. The lesions are found in 100% incidence 16 days after immunization, and at 7 weeks the disease is severe and extensive in all animals. This has persisted as long as 6 months, at the end of which time some decrease in severity has been found. In studies on mechanisms involved in this autoimmune disease, antibody levels, as well as tests for delayed hypersensitivity to thyroid extract, have been followed. Findings thus far suggest that the presence of the disease is correlated with delayed hypersensitivity, but not necessarily with circulating antibodies. Inasmuch as auto-allergy is felt, at present, to be the underlying process in several human diseases, information derived from these studies as experimental models can be of potential importance.

TRAUMA IN AMOEBIC INFECTIONS

In continued studies aimed at an understanding of the role played by bacteria in aiding Endamoeba histolytica to establish infection and produce lesions in the intestine, efforts were directed at trying to determine whether trauma or damaged mucosa were essential. As we reported previously, it was possible to get some chronic-type lesions in the intestinal wall of germfree guinea pigs with amoebae that were prepared by techniques which apparently resulted in more vigorous organisms than had been used in the past. However, most of the lesions seemed to occur at the site of the inoculation. Recently we have tried inoculations via the terminal ileum whereby the amoebae were delivered into the cecum at some distance away from the puncture wound. Under these conditions

the number of germfree animals developing lesions in the cecum was significantly reduced. Thus, at least in the case of the type of lesion obtained in the absence of a flora, trauma or some frank tissue break appear to be necessary for the amoebae to establish infection.

SECOND GENERATION GUINEA PIGS Some further studies on the biology of germfree guinea pigs were conducted. The lack of bacteria seems to have more profound and diverse deleterious effects on guinea pigs than on other laboratory animal species that have been reared. We were able to obtain two litters of second generation germfree animals. Only rarely and recently has this been possible. The growth rate and general physical condition of these young was better than has been experienced with Caesarean-derived animals. The fact that they received maternal milk perhaps may explain their better performance.

GERMFREE CHICKENS In further studies to ascertain activities of the intestinal flora, it was found that 6-week-old germfree chickens did not contain valeric acid in their intestinal tracts. This substance is a common constituent of gut contents in conventional chickens and, therefore, these findings indicate that it arises from microbial fermentation. Of further interest was the fact that chromatographic analyses of gastro-intestinal material from germfree chickens failed to reveal any fermentation acids in the crop. However, lactic acid was demonstrated to be present throughout the lower GI tract in amounts which are normally found in conventional birds. This rather unexpected finding in the absence of a demonstrable flora must be explored further.

LABORATORY OF INFECTIOUS DISEASES

The work of the virus and epidemiology sections continued to be aimed at better definition and understanding of numerous human and animal virus infections, particularly of their roles as etiologic agents in respiratory disease, cancer and birth defects. In other words, the chief concern is to find the true natural history of viruses, and define their importance in various disease states. In order to do this it has been necessary to develop appropriate tests and survey methods and in 1961 we were able to achieve comprehensive surveys of viral infections not only in man but also special studies of domestic and commensal animals. We have continued field and clinical studies in collaboration with research personnel of the Bureau of Medicine and Surgery, U. S. Navy; D. C. Children's Hospital Research Foundation; the D. C. Welfare Department; the National Institute of Neurological Diseases and Blindness; the National Cancer Institute; and the Laboratory of Clinical Investigation of the National Institute of Allergy and Infectious Diseases.

RESPIRATORY VIRUS DISEASE Previous annual reports and publications have documented the key role of LID scientists in the discovery of adenoviruses, parainfluenza viruses and respiratory syncytial viruses, and in defining their importance in causing respiratory illnesses, especially in children. In 1961 the role of these agents in causing 35 to 45 percent of the acute respiratory illness of infants and children was

confirmed. In addition, the contribution of the enteroviruses (Coxsackie and ECHO viruses) to the acute respiratory syndrome was partly elucidated. Studies of outbreaks of Coe virus (Coxsackie A-21)in military recruits, Pett (Coxsackie A-24), Coxsackie B 3 and 5 viruses suggest that these enteroviruses (and new unclassified ones similar to the British Salisbury viruses) will soon be found to contribute significantly to the overall respiratory disease problem.

EATON AGENT IS PPLO

The most significant finding since the beginning of our studies of Eaton's "virus" (Primary Atypical Pneumonia) was the demonstration in 1961 that it is not a virus but a PPLO (pleuropneumonia-like organism) which can be grown in synthetic media.

This discovery is exciting for three reasons. First it opens the door for the development of much simpler techniques for diagnosing primary atypical pneumonia. Secondly, it provides a ready source of material for preparing a preventive vaccine, and thirdly, it suggests that additional serologically different PPLOs can be expected to turn up as causes of acute respiratory illnesses.

The fact that pneumonia due to the Eaton agent responds readily to tetracycline therapy and the fact that PPLOs are known to be generally very susceptible to broad spectrum antibiotics furnished previously undreamed of possibilities in the therapy of respiratory illnesses.

ENTEROVIRUSES IN ACUTE RESPIRATORY DISEASE

The enteroviruses (Poliovirus, Coxsackie and ECHO viruses) are well known as causes of certain specific illnesses such as poliomelitis, herpangina, pleurodynia, aseptic meningitis and myocarditis. Less well appreciated are their importance in causing acute upper respiratory illnesses. It is evident from our long term studies of Junior Village children (where we observed nearly 2,000 persons infected with many different enteroviruses) that acute respiratory illnesses often with fever represent the most common clinical manifestations of most enteroviruses. Recently using more sensitive tissue culture isolation procedures, we have encountered numerous additional viruses having the properties of Coxsackie A viruses. the most common group of similar isolates are related to Coxsackie A-24 Pett virus which is related to Coxsackie A-24 virus.

Information on the role of enteroviruses as an etiologic agent of adult respiratory illness, particularly the common cold syndrome has been derived chiefly from outbreaks of ECHO 28 (2060 virus) reported at different times chiefly from Chicago by Magabgab several years ago and in 1961 by Hamre and the outbreaks of Coxsackie A-21 in Marines observed last year. It appears that viruses can be recovered from about 20 to 25 percent of adults with mild respiratory illnesses, thus providing new dimensions for study of the "common cold."

ANTIBIOTICS IN ACUTE RESPIRATORY DISEASES

We completed studies on the value of tetracyclines in the treatment of primary atypical pneumonia due to Eaton's (PPLO) agent in adults and on the value of oral penicillin in preventing acute febrile respiratory illnesses in children. The first study of pneumonia which was carried out in Marine recruits at

Parris Island (in collaboration with the Bureau of Medicine and Surgery, U.S.N.) showed that declomycin, a synthetic tetracycline, was very effective in the treatment of primary atypical pneumonia, reducing significantly the duration of fever, pneumonitis and hospitalization.

The daily use of oral penicillin for nearly a year in a proportion of children at Junior Village failed to significantly alter the overall illness rate despite the fact that hemolytic streptococcal infections were virtually eliminated from the throats of those in the test group. Only during brief outbreaks of several typable hemolytic streptococci was it possible to show any measurable beneficial effects from the drug. No effect was observed on viral infections.

RESPIRATORY VIRUSES IN HUMAN VOLUNTEERS

Many studies of numerous respiratory agents (viruses and PPLO) in volunteers are scheduled to be carried out in cooperation with the Laboratory of Clinical Investigation and the Federal Bureau of Prisons. To date studies of the pathogenesis of several parainfluenzas, RS, and Coe (Coxsackie A-21) viruses, and Eaton's PAP agent grown in tissue cultures have been completed. All were found to cause acute respiratory illnesses. Eaton's agent produced not only primary atypical pneumonia but also middle ear infections; in the latter case providing one of the first specific clues to the cause of nonbacterial serous myringitis.

One of the more interesting findings from the volunteer studies was the fact that many of the virus infections produced illnesses despite the presence of pre-existing antibodies; but the antibodies also were responsible for the general mildness of the illnesses observed.

Studies in volunteers of Coxsackie A-21 virus (a Coe-like strain isolated from a military recruit) revealed that growth of this enterovirus was almost entirely restricted to upper respiratory tract. This is interesting since other Coxsackie A-21 strains are commonly found in the stools of children. Carefully designed volunteer studies provide a wealth of high order information on host-parasite relationships virtually unattainable in any other way. Hence they will be continued and expanded.

VACCINE DEVELOPMENT

One of the chief purposes of the volunteer study program is to test live viruses for attenuation and for inclusion as candidates in experimental vaccines. Of course, killed vaccines can also be tested in volunteers - the efficacy of the vaccine can be measured by challenge with a pathogenic variant of the virus as previously done by LID scientists in developing the first effective adenovirus vaccine.

SERO-EPIDEMIOLOGY OF HUMAN VIRAL DISEASES

During 1961 antigens against more than 100 common viruses (6 myxoviruses, 28 adenoviruses, RS, 3 reoviruses, 59 enteroviruses, and 10 mouse viruses) were evaluated in the micro-CF and micro-HI test systems. This program represents collaboration between the cerebral palsy study group of NINDB and all of the research units in the Virus and Epidemiology Sections of LID.

Extensive serologic-epidemiologic surveys are now in progress on the prevalence of antibody responses against more than 100 viruses in women giving birth to abnormal babies, and in children and adults with acute respiratory illnesses, neurological diseases, and cancer. The prevalence of specific adenovirus and myxovirus antibodies in sick children at Junior Village and Children's Hospital is now under study, thus adding a new dimension to our knowledge of the natural history and pathogenesis of these agents.

Similarly serological surveys of these populations have revealed new and wholly unexpected information on the enteroviruses. Previous data from Junior Village which suggested that enteroviruses were important causes of acute respiratory illness, and that this may be the most frequent illness caused by enteroviruses, was confirmed by serologic studies of patients from D. C. Children's Hospital. These tests showed in 1961 that enterovirus infections in children with acute respiratory disease were eight times as frequent as in age-matched control children without respiratory illness.

CANCER VIRUSES Studies of animal cancer viruses have proceeded satisfactorily both in the field and in the laboratory. Perhaps our most important function is to focus attention on the importance and feasibility of studying the occurrence and behavior of cancer viruses in nature, to help develop laboratory tools which make such studies possible, and to continue to help define the extraneous viruses in cancer virus study systems which are producing almost insuperable obstacles to the development of reliable information about cancer viruses.

NATURAL HISTORY OF POLYOMA VIRUS The natural cycle of polyoma virus was found to be well established in Mus musculus living in farm industry ecologies in Maryland and in Indiana. There is extensive evidence that the age-old association of the house mouse first mentioned in relation to grain storage in the Mediterranean area (recorded in ancient Egyptian and Greek records and the Old Testament) now persists in modern grain storage facilities, on farms and in central depots all over the world. Although Mus musculus is now indigenous to all parts of the world, it originated in southern Asia and the Middle East, from whence it was carried along lines of trade to other parts of the world reaching northern Europe and England by 900 A.D. and becoming well established in the New World only within the last two centuries. Studies in southern England and nationwide surveys in the United States show that infestation of grain storages are extensive, almost universal, and frequently very intensive. For instance, a recent 29 state U.S. survey revealed an average count of 80 mouse pellets per peck (about 15 lbs) of shelled corn.

LID studies in the field to date show that polyoma virus infection could be demonstrated in Mus musculus on 4 of 15 Maryland dairy farms, in 6 of 6 Maryland cereal grain mills and 3 of 4 Indiana grain mills. Polyoma virus was isolated from cereal grains used chiefly for livestock feed (but also for laboratory mouse and human consumption) wherever such grains showed objective evidence of mouse contamination and where the mice were found to be positive for polyoma. This was not surprising, since infected mice are known to excrete large amounts of virus for long periods in the urine and infestation with polyoma-positive mice was very heavy in most of

the ecology studies. Thus, as reported last year, the polyoma cancer virus is widely distributed in all the ecologies in which commensal house mice live - in laboratories, in production colonies of laboratory mice, in densely populated urban tenements, and in rural environments.

EXTRANEOUS VIRUSES IN CANCER-VIRUS STUDY SYSTEMS

This problem has if anything become worse. Additional viruses were found in production colonies of laboratory mice, including PVM, new strains of hepatitis and Riley's plasma agent. For instance, Riley's agent, together with reovirus 3 and hepatitis virus turned up successively in our passage lines of Moloney's leukemia.

On the credit side we have been able to develop satisfactorily in vitro survey tests for mouse hepatitis, PVM, Theiler's virus, and LCM, thus adding these to our test battery which already included tests for polyoma, K virus, mouse adenovirus and mouse salivary gland virus. Thus by working with commercial producers of laboratory mice, we hope to achieve rather soon supplies of mice that are free of infections with these agents.

Immunological studies of mouse reoviruses showed them to be indistinguishable from those isolated previously from man, cattle and monkeys. An interesting sidelight was the demonstration that Stanley's encephalohepatitis virus is identical with reovirus type 3 and that the various other hepato-encephalitis agents isolated from mice and described by Cheever and others are serologically related to MHV and other strains of hepatitis virus.

SPF AND GERMFREE MICE IN CANCER RESEARCH

The obvious answer to the background noise problem is the use of susceptible SPF (specific pathogen free) mice and germfree mice. The main difficulty with the latter is that they simply are not available in large numbers. The SPF mice as suggested above, are of value only when they can be kept in isolated virus-proof quarters, which currently are not available in our virus building.

We have tested two methods of isolation-both with considerable success. The first method made use of isolated house trailers which are chemically sterilized between experiments. These have been very useful for studies of polyoma virus in such field specimens as mouse tissues, excreta and mouse contaminated grain.

Recently, successful experiments have been performed in the Lobund plastic isolators. Although these require much attention and high caliber animal attendants, they may well be the answer to the production of pedigreed, clean and certified cancer virus pools - a prime requirement in cancer virology just as it is for respiratory virology.

FUNGUS DISEASE

Mycology studies have followed a multidisciplinary and multifacted research program. In the field the work is focused on isolation and identification of new fungi and on ecologic studies on various fungi known to be pathogenic for man. The studies of new fungi are done in collaboration with the hospitals and diagnostic laboratories;

the ecologic studies in collaboration with the Rabies Control Unit, Trinidad, West Indies. Additional field programs are planned. New antimycotic drugs and antibiotics are tested for efficiency and safety, in collaboration with the Medical Physiological Bacteriology Section of LID and with the Laboratory of Clinical Investigation. The clinical and field studies are supported and supplemented by basic work in laboratory on the physiology, toxicity and immunology of pathogenic fungi. The search continues for serological methods capable of recognizing mycotic infections in humans and in various animal hosts, and for improved techniques designed for growing and identifying fungi. Histoplasma capsulatum was isolated in Washington, D. C. for the first time from a congested urban area, from soil adjacent to houses in Trinidad, W.I., which harbor bats, and from soil under roosting sites of starlings.

PENICILLINASE

A highly interesting observation concerning staphylococcal penicillinase has been made. It seems probable that this enzyme is a non-specific cyclic peptidase, splitting various cyclic peptides to straight chain compounds -- this observation offers at least a partial answer to the hitherto disturbing question of the role and function of penicillinase in bacteria in their natural habitat. Under normal ecological conditions, it is highly unlikely that bacteria ever come into contact with penicillin, hence the value of the possession of penicillinase to the total economy of the cell has been dubious. By offering the possibility that penicillinase is primarily a specialized peptidase, its role is conceivably enlarged to a more important function in cellular metabolism.

ANTIBACTERIAL MARINE WATERS

Significant observation have been made on the problem dealing with the antibacterial activity of marine waters. It is now quite clear that the activity is due to a large organic molecule with molecular weight greater than 10,000 rather than due to the inorganic ion content of the sea water. This observation, obtained as a result of exhaustive dialysis experiments, settles in large part a controversy of 75 years amongst biological oceanographers. Partial isolation and purification of the active factor has been obtained. Further, the activity seems to be quite specific for gram-positive bacteria, thus offering an attractive hypothesis in explanation of the fact that the vast majority of bacterial genera in the oceans are gram negative. The activity is always present in ocean water, varying only quantitatively and this finding answers in part the hitherto disturbing observation that activity of raw sea water varies temporally and seasonally. From the point of view of isolation and purification of an antistaphylococcal factor, particularly active against penicillinase-producing staphylococci, this is, of course, decidely advantageous.

STAPHYLOCOCCUS IRON METABOLISM

Highly significant observations have been discovered this year dealing with metabolic differences occurring between iron-deficient and iron-sufficient strains of Staphylococcus aureus. The degree of iron depletion or enrichment was controlled by the ratio of free Fe to iron-bound siderophilin in the medium of growth of the organisms. Using radioactive tracers, it is now quite clear that iron-deficient cells lack the ability to metabolize the six carbon of glucose when compared with iron-sufficient cells and further that the deficient cells also have decreased ability to incorporate carbon into protoplasm. These

observations are a highly important beginning in a project designed to understand the biology of staphylococci. Since production of the various staphylotoxins presumably mirrors the overall metabolic processes of the cells and since, as indicated above, iron plays such a highly important role in metabolism, the hope remains that by juggling iron availability in vivo (and this can be done by proper use of siderophilin), one will be able to render invading staphylococci less toxic.

In the development of the above thesis, it has been essential to devise methods for purification of physiologically active siderophilin and for assaying such activity. This has finally been done by various ingenious biochemical techniques and the problems can now be attacked forthrightly.

CELL DIVISION IN STREPTOCOCCI

Study of the M antigen of Group A, beta-hemolytic streptococci has yielded a surprising but highly important dividend of basic significance. A controversy which has been going on for decades over whether synthesis of the cell wall in dividing bacteria occurs by random intercalation or at specifically polarized growing loci has been all but settled, at least for the Group A streptococci. Using, alternately, periods of growth in culture media with and without fluorescein-labelled homologous anti-M protein, the issue has been definitely decided in favor of the latter hypothesis, i.e., polarization. The observation is of significance to all studying cytokinesis and morphogenesis in bacteria and fungi as well as to those interested in cell-wall metabolism in general. Interest, of course, in cell walls has been high in recent years since many important inhibitors, including penicillin, presumably act by virtue of inhibition cell-wall synthesis.

Further improvement of the "long-chain" test for streptococcal type specific antibody has been made and its simplicity and accuracy appear likely to make it a most valuable tool for the answering of many clinical and epidemiological studies. Currently, it is being used in the study of pre- and post-isolation sera from a type 12 streptococcal outbreak among institutionalized nursery-school-age children.

The mechanism of the long-chain reaction has been under investigation and the possibility exists that the phenomenon is a form of agglutination requiring bivalent antibody and may not be enzymatic as suggested by other workers.

ANTIBACTERIAL SUBSTANCES IN MOLLUSKS

Of vast potential import is the isolation, characterization and partial purification of antibacterial and antiviral substances from mollusks, particularly oysters. Two fractions, decidedly different in behavior on columns, but both presumably glycoproteins, have been found to be active against bacteria, including staphylococci and viruses (polyoma and influenza). The significance of this is obvious for an effective chemotherapy of small virus infections is of great moment.

HYDROGENOMONAS Marked advances have been made in an understanding of the electron transport of Hydrogenomonas and as a consequence, relying on the thesis of comparative biochemistry, the main energy-yielding systems of mammalian tissues. The system in the autotrophic Hydrogenomonas is ideal for these studies since it is, in distinction to systems in all other forms studied, at least partially soluble. This fact obviates the extreme difficulty of working with particulate and hence highly complex material. A further indication of the relative simplicity of the complex in this organism has been the finding that it contains only one cytochrome. It now seems obvious that flavoproteins are active in this system in the transport of elutions and a judicious thesis can be presented for the formation of reduced pyridine nucleotides via the hydrogenase system. However, a new intermediate, different from others heretofore described, apparently is involved in the formation of reduced pyridine nucleotide. This, of course, has vast theoretical ramifications and would indicate important differences between bacterial and human energy-yielding systems and might well indeed be a basis for selective attacks on bacteria by antibacterials.

LABORATORY OF CLINICAL INVESTIGATION

Summary.. 1

10 (c) - Office of the Chief........................... 8
11 (c) - Mechanisms of Fever........................... 9
12 (c) - Respiratory Viral Diseases.................... 14
13 (c) - Antimicrobial Drug Therapy.................... 18
14 (c) - Staphylococcal Diseases....................... 23
15 (c) - Hepatitis and Mononucleosis................... 24
16 (c) - Aseptic Meningitis............................ 25
17 (c) - Allergy-Immunology............................ 26
18 (c) - Enteric Diseases.............................. 31
19 (c) - Clinical Investigations on Helminthic Diseases.................................... 33
20 (c) - Clinical Investigations in Protozoan Infections and Diseases....................... 34
21 (c) - Cystic Fibrosis of the Pancreas............... 44
22 (c) - Basic Biochemical Studies..................... 45
23 (c) - Systemic Fungal Diseases...................... 49
24 (c) - Sarcoidosis................................... 53
25 (c) - Pyelonephritis................................ 55
26 (c) - Urinary Tract Viruses......................... 56
27 (c) - Biochemical Studies on Staphylococcal Cell Walls....................................... 59
28 (c) - Pathogenesis of Viral Infections.............. 60
29 (c) - Mid-Line Granuloma and Wegener's Granulomatosis.............................. 63
30 (c) - Studies on Immediate and Delayed Sensitivity to Simple Chemicals and Protein.................................... 65

Summary of Research Progress
Calendar Year 1961
Laboratory of Clinical Investigations

The program of the Laboratory has been enlarged by the development of projects in viral respiratory disease, immunology, mechanism of fever, and malaria, without sacrifice of any previous activity. In contrast to previous years when optimum bed occupancy was achieved only with difficulty, the daily census has been maintained near 100 per cent by reason of the volunteer program, and there is now a waiting list for other cases which permits a more careful selection of those to be admitted.

The number of professional personnel remains about the same. Dr. Henry Beye was appointed Director of the MARU operation in Panama and left in the Spring. Dr. Robert Couch joined the Laboratory in September. Dr. Couch was a Clinical Associate in the Cancer Institute for two years and then was on assigned duty at Vanderbilt University Hospital for two years before joining our staff. He has been temporarily assigned to work with Dr. Robert Chanock in the Laboratory of Infectious Diseases (NIAID-72). The concept of close collaboration of the clinical program with basic science laboratories has been extended this year so that, in addition to Dr. Couch's assignment to a virology laboratory, two other men have been similarly assigned to work with Dr. Karl Johnson (NIAID-72B). All of these men are concerned with the virus-volunteer research program and by reason of their laboratory assignment follow the clinical problem into the laboratory. In addition, three men are assigned to the Laboratory of Immunology working on problems of both clinical and basic interest (NIAID-17(c), 145, 145A, 156). Continuing a previous practice one man is working part-time in Dr. Emmon's mycology laboratory (NIAID-76B) and he is also affiliated with the clinical mycology program. One other worked on malaria with members of the Laboratory of Parasite Chemotherapy (NIAID-130A).

Pediatrics:

A difficult decision to de-emphasize the program in pediatrics has been made this year. It will be recalled that a small pediatric service had been maintained here for some years, primarily concerned with research in cystic fibrosis of the pancreas. Despite considerable interest and industry on the part of the pediatric clinical associates, little research progress resulted from this investment. There were numerous patients and excellent nursing and hospital facilities, but the program suffered from the lack of senior personal supervision. Reference was made in a previous report to the difficulty of obtaining mature, qualified men to head sections, and this was especially true of pediatrics. Several men were approached concerning the position and all declined to consider it seriously. There seemed to be some hesitancy on their part in accepting government employment and it was further observed that the salaries offered were about the same as they were then receiving. Moreover, there was little hope of further increase in the NIH salary after accepting an appointment.

The General Problem of Professional Recruitment:

Almost any professional observer will agree that the NIH offers one of the most ideal opportunities for a career in medical research, and yet we recurringly discover that we cannot tempt a man away from a position in a medical school by offering him these privileges with a salary approximating that which he receives at the school. The reasons for this, I believe, are first, that being a member of a university faculty is an inspiring and satisfying experience. Second, men rarely change positions unless it constitutes a promotion, i.e. significant increase in salary as well as scientific opportunity. (I detect in government personnel officials the unrealistic attitude that if we match the salary a man is getting elsewhere, he should be pleased to come and work at our excellently equipped institution.) Third, as described in last year's report, promotion of good men in medical education may proceed very rapidly, at rates vastly in excess of what may be accomplished in government. We have an excellent opportunity for young investigators and we have several, but when they reach the intermediate and senior stages of development, they can advance farther, faster outside of government. This has resulted, I believe, in a deficiency of well-qualified men in many areas at intermediate and senior levels at NIH. I am also afraid that one of our best advertised virtues, the security of employment, leads to a selection of personnel who do not have the broad perspectives and aggressive scientific interest the institution needs. The NIH ought to deal only in talented men and to do this some system to attract and keep them must be established.

Finally, I believe that the low salaries paid the permanent staff make conspicuous out of proportion some of the restrictions on travel, participation in meetings, committees, scientific organizations, scientific writing, and consultations. These restrictions are further emphasized by a high degree of personal privilege in all of these matters among university faculties; a state of privilege that should not be confused with academic freedom which is present at NIH without serious limitation.

NIH is in the market for a number of talented young men each year, some of whom we would like to keep as permanent staff members, and in this purpose we are constantly in competition for personnel with medical education, industry and private practice. I believe we are losing the race to a painful extent because we are underpaying intermediate and senior staff. Further, I believe that if we could increase salaries in this group by 20-25% our personnel problems would largely disappear until the market for scientific personnel increases another step.

Scientific Program:

During the year, the 18 professional members of the staff and their collaborators engaged in 52 different projects, about one-third of which have been completed.

Viral infection of volunteers. The largest program of the laboratory has been studies of viral respiratory disease in prisoner volunteers. During the year, the organization and function of this activity has become more efficient. A total of 189 volunteers have been inoculated with one of 14 different respiratory viruses or the Eaton agent. The principal purpose of

these studies has been to determine the capacity of the agents to cause illness and to study the virological and immunologic response to inoculation. The viruses tested were, with exception of influenza, suspected of causing significant amounts of respiratory viral infection, but their role had not been defined. Thus, a determination of their capacity to cause illness represented an important step in assessing their role in the total picture of respiratory disease in the general population.

A classical type of moderately severe viral influenza has been produced in susceptible volunteers while a milder disease or no illness occurred in those with specific antibody. Illness and viral excretion began by the second day after inoculation and virus was still present in some cases 5 or 6 days later, and after recovery from illness. These studies have demonstrated a type of infection and illness which lends itself to many kinds of clinical study.

Despite many close biologic and biochemical similarities it was found that Coxsackie A-21 (the Coe agent) caused an acute, febrile flu-like illness while Coxsackie A-24 (the Pett virus) caused no illness. Virus was repeatedly isolated from the throat of volunteers given A-21, while A-24 was found only in rectal swabs. This surprising difference will be studied further.

Several higher-type adenoviruses have been shown to produce disease when inoculated by cotton swab into the conjunctiva beneath the lower lid but not when given intranasally or by spray into the throat. In all situations, however, viral infection occurred with both throat and anal swabs positive. The higher type agents appear to be less virulent than lower type adenoviruses, as judged by comparison with earlier studies by Huebner.

Reo-viruses obtained from human sources failed to produce illness although viral infection occurred in susceptibles. It is planned to do similar studies with apparently identical viruses isolated from cattle. Recently, a common cold syndrome with rhinorrhea, malaise, and in some cases, fever, has been produced by three new and presently unclassified enteroviruses. The incubation period was only a few hours.

A joint venture is currently underway with investigators at Ft. Detrick, Maryland. They are preparing several viral agents in small particle aerosol and with their equipment, cautious studies of the effect of introducing inocula of small particles deep into the bronchial tree will be made. It is also planned to test the effect of an adenovirus inhibitor of biologic origin in treatment of infection. (This is distinct from interferon, and there is some prospect for future studies of this substance as well.) Other results of the studies in volunteers will be described by Drs. Chanock and Johnson (NIAID-72B).

Bacteriology. Two new penicillin derivatives have been tested in staphylococcal infection. The agents, Prostaphlin, manufactured by Bristol Laboratories, and SKF No. 12141, manufactured by Smith, Kline & French, are similar in that they are substantially resistant to destruction by penicillinase, they are significantly more active on a weight basis against sensitive staphylococci than dimethoxy-phenylpenicillin, the first of the penicillinase

resistant compounds (see last year's report), and they are active after oral administration. Limited clinical studies have revealed that these agents are effective in the treatment of staphylococcal infection, including those resistant to older forms of penicillin. Biochemical studies on these compounds have also been made and will be described elsehwere in the report.

Last year Dr. G. P. Gladstone from Oxford was a visiting scientist with Dr. George Williams in clinical bacteriology. During his stay he extended his studies on the measurement of staphylococcal anti-leukocidin as a basis for detection of acute infection with this organism. These studies were in part made on material from cases of cystic fibrosis with pulmonary staphylococcal infection and on other cases from our clinical service. Meanwhile, in Czechoslovakia, studies of staphylococcal leukocidin as an immunizing agent were underway which now reveal a significant protective effect of this material. Recently, Dr. Stuart Mudd of Philadelphia has obtained a promise of some of this vaccine from Czechoslovakia and has asked LCI to participate in its evaluation. This program will begin early in 1962.

Studies were made of 29 chronic salmonella carriers and it was revealed that gallstones present in every case contained salmonella (most were _S. typhosa_). When these stones were incubated in high concentrations of antibiotics, salmonella persisted and grew in subsequent cultures not containing antibiotics. Consistent with these findings was the observation that antibiotic treatment alone was not effective in curing the carrier state. On a basis not yet fully explained, was the further observation that cholecystectomy alone was often not effective in curing carriers, suggesting infection in sites other than gallstones which were the basis for perpetuation of the carrier state (a few instances of positive cultures from liver tissue were found). Finally, it was found that lasting cure occurred if antibiotic treatment was combined with cholecystectomy. It was estimated that over 300 cases and 3 deaths from typhoid occurred in contacts of this group of patients.

Malaria. Following demonstration of human infection with the simian vivax-type malaria, _Plasmodium cynomolgi bastianellii_, several prisoner volunteers infected with this agent, and others inoculated with human vivax strains were studied at the Clinical Center. In addition to the usual parasitologic determinations, radioactive chromium survival tests, excretion of adrenal steroid metabolites, liver function, a number of other parameters were measured. Among other findings was the observation that a significant reduction in serum haptoglobin concentration took place in association with hemolysis of the red blood cells.

The most significant observation, however, was the demonstration of fluorescent stainable malaria antibody appearing approximately two weeks after onset of illness and reaching a peak about one month later. Titers as high as 1/5120 were frequently reached. Following recovery the titers slowly decreased. In connection with these studies, it was found that the concentration of circulating gamma globulin in the malaria patients increased during infection, and the highest values of gamma globulin coincided closely in time with the occurrence of the highest titer of malaria antibody.

Mechanisms of fever and host response. A new program begun this year

has been that of investigation of host response to fever, and causes of fever and related phenomena in certain febrile diseases of obscure origin. The studies are presently centered around the illness known as familial Mediterranean periodic fever or familial polyserositis. It is an inherited disease of certain persons of Jewish, Armenian and sometimes of Turkish descent. It is characterized by many years of progressive, disabling attacks of non-infectious fever and peritonitis unrelieved by any presently known treatment. Studies here have been concerned with the effect of the recurrent fever on mechanisms of heat loss when the patients are stressed in a low temperature chamber. It appears now that patients with polyserositis have lost some responsiveness to cold stimulation as compared with normals. Studies of gastrointestinal motility, metabolism of adrenal steroids, fibrinogen and platelets, and of the effect of endotoxin injection are underway. A number of interesting but as yet incomplete results have been obtained. There is some evidence this may be an endotoxemia of uncertain origin.

Another study as yet incomplete is that of the treatment of midline granuloma with high doses of deep x-ray. Persons with this unusual illness often succumb after a painful illness characterized by a generalized destruction of the midline area involving the nasal turbinates, walls of sinuses and orbits and adjacent soft tissue, and there is evidence in some cases that the disease may be a hypersensitivity reaction. Results in three patients treated with high doses of x-ray reveal pronounced, immediate improvement, but periods of observation after treatment are still too brief for a decisive evaluation.

Fungus disease. The program in systemic fungus infection has continued in a vigorous way. A number of clinical reports have been made of treatment of various kinds of fungus disease with amphotericin and the new agent of Hoffman-La Roche, X5079C. Both drugs are extremely useful in treatment of fungus disease as described in the following table:

	Amphotericin	X5079C
Blastomycosis	Good	Good
Histoplasmosis	Good	Good
Sporotrichosis	Good	?Good
Cryptococcosis	Good	Doubtful
Coccidioidomycosis	Slight	Doubtful
Candidiasis	Moderate	Doubtful

Since amphotericin causes significant renal toxicity and local irritation, especially when introduced into the spinal canal these features of treatment have been studied. It was found that a persistent renal tubular lesion characterized by prolonged depression of para-amino hippurate clearance is caused by amphotericin. Furthermore, recent studies of biopsies of kidney from patients under treatment reveal necrosis of glomeruli. This is an unusual renal lesion and is under detailed investigation in dogs. So far no effective means has been found to control the irritative effects of the drug on the central nervous system after intrathecal instillation. The nerve damage encountered has been so severe that local use of the agent in meningitis (cryptococcal) will henceforth be administered only to those cases in which

it is considered an absolute essential.

X5079C was found to cause a prompt rise in bromsulfalein retention despite only slight evidence of interference in other kinds of liver function. This received careful examination in dogs and the effect is now revealed to be a competition of the antibiotic for the excretory route for bromsulfalein. The exact mechanism has not been determined. Prolonged treatment with the agent causes some liver damage but it appears to be sufficiently slight to permit therapeutic use of the drug.

Biochemistry. The work of the Section on Biochemistry and Immunochemistry in the past three years on the problem of penicillin-resistant staphylococcal infections has developed into two distinct problems. The first phase of the investigation dealt with the nature of the resistance of staphylococci to penicillin. It was established that naturally-occurring pathogenic strains of Staphylococcus aureus which are resistant to penicillin, are resistant by virtue of their containing an enzyme, penicillinase, which rapidly destroys penicillin G. A series of papers have been published on the biochemical properties of this enzyme as it occurs in S. aureus and on its role in penicillin resistance.

The second phase of the investigation has been concerned with the many new penicillins that have become available in the past two years as the result of a technologic breakthrough in the field of penicillin chemistry; namely, the ready availability of 6-aminopenicillanic acid as the immediate precursor of a wide variety of penicillins previously unknown. When it was determined that resistance of staphylococci was based on their ability to destroy penicillin G at a rapid rate, it became evident that a possible solution to the problem would be to find a modification of penicillin which would be resistant to the destructive action of penicillinase and yet retain antibiotic activity. When 2,6-dimethyoxyphenylpenicillin was given to the Laboratory for clinical trial, it was very soon found that this new penicillin met the essential biochemical criteria and offered therapeutic promise. Our laboratory, by virtue of being the first to obtain staphylococcal penicillinase in large amounts in concentrated and purified form, was able to establish the absolute rate of destruction of the new penicillin by staphylococcal penicillinase and show it to be more resistant than penicillin G by a factor of about 200 to 1. The consistently good therapeutic results obtained with this drug on extensive clinical usage in the past year have tended to verify the conclusions drawn from the laboratory findings.

Similar techniques have enabled us to evaluate all the new penicillins which have been offered to us for clinical trial including an isoxazolyl penicillin which is not only resistant to penicillinase but is also sufficiently stable to acid to permit its oral use. Such a penicillin would have an advantage over 2,6-dimethoxyphenylpenicillin which is not effective after oral administration and must be given intramuscularly or intravenously.

Biochemical investigations are underway on still another new type of penicillin, gamma-amino-benzylpenicillin, which for the first time permits a penicillin to be used as a broad spectrum antibiotic since this compound destroys gram negative organisms in addition to the usual gram positive species.

Studies are under way on the many interactions between penicillin, the infectious agent, and the host system, in order to elucidate some of the underlying mechanisms involved in such phenomena as antibiotic activity, penicillin resistance, and penicillin allergy.

Immunology. The development of a clinical immunology section has provided the Laboratory of Clinical Investigation with an increasing number of laboratory techniques to support our clinical studies. The immunoelectrophoresis technique, other gel diffusion methods, and the fluorescent antibody techniques have been used to study the serum of patients with the nephrotic syndrome, lupus erythematosus, and many other of the disease entities under study by other members of the laboratory.

A group of 60 patients with the nephrotic syndrome studied over the past eight years are being followed to evaluate the effect of steroid therapy in these diseases. Similar studies are being made with a group of patients with lupus erythematosus and in addition, these patients are being evaluated for the degree of skin test hypersensitivity to autologous leukocytes. Since the therapy with steroids is often not completely effective and since initial results suggest that those purine antimetabolites which apparently act by suppressing antibody production are effective in the treatment of certain diseases associated with auto-immune phenomena, a study of the effectiveness of these drugs in the nephrotic syndrome and lupus erythematosus has begun.

Two years ago it was discovered in this laboratory that mice injected with Freund's adjuvant and certain antigens developed chronic ascites containing a high titer of antibody to the administered antigen. Later it was shown that a certain number of the animals, and this was strain-related, developed plasma cell tumors which were rapidly fatal. Further, it was observed that the tumor-bearing mice were poor producers of antibody. These tumors have been found to produce predominantly one or another types of globulins (alpha, beta, gamma) and currently studies are underway to measure the relative responsiveness to antigenic stimulation of mice with these tumors.

Serial No. NIAID - 10 (c)
1. LCI
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Office of the Chief

Principal Investigator: Dr. Vernon Knight

Other Investigators: None

Man Years (calendar year 1961):

Total: 7.5
Professional: 1.0
Other: 6.5

Project Description:

Objectives:

To develop, direct and coordinate the program of the Laboratory, as defined in the individual research projects; where necessary, to redirect individual projects to meet current needs and advances in the field; to align clinical projects with patient availability; to provide an unexcelled standard of patient care for patients utilized in research.

Methods Employed:

Organize available staff and recruit qualified personnel, both professional and sub-professional, to develop the program and carry out its aims; close liaison with area medical societies, institutions and individuals for referral of patients whose diagnoses fall within the active or proposed disease research categories; the highest standards of patient care are maintained by selection of qualified physicians; the development of necessary policies and continued close and direct supervision of patient care activities; professional consultant services to other institutes in the area of infectious diseases; continued guidance of research projects undertaken by younger investigators; maintenance of staff morale.

Part B included Yes /_/ No /X/

Serial No. NIAID 11 (c)
1. LCI
2. Bacteriology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Mechanisms of Fever

Principal Investigator: Dr. Sheldon M. Wolff

Other Investigators: Dr. Stanley Ward, LCI, NIAID
Dr. Sanford H. Stone, LI, NIAID
Dr. Howard C. Goodman, LI, NIAID
Dr. Fred Gorstein, LGFR, NIAID

Cooperating Units: Dr. E. Buskirk, NIAMD
Dr. Ronald H. Thompson, NIAMD
Dr. N. Raphael Shulman, NIAMD
Dr. Leonard Laster, NIAMD
Dr. Roger Black, NIAMD
Dr. Bernard Kliman, NHI
Dr. George Bryan, NHI

Man Years:

Total: 1.3
Professional: 1.3
Other: -

Project Description:

A small group of selected patients is being intensively studied on the wards and in the laboratory. The patients generally can be divided into two major groups, one group are those patients with familial Mediterranean fever (Familial Polyserositis) and the second group are patients with a variety of disorders characterized by recurrent fever.

Subproject I: Familial Mediterranean Fever (FMF).

Objectives:

1. The role of pyrogen in the febrile sera of patients with FMF.

2. To ascertain if the afebrile sera of FMF patients decreases the lethality of endotoxin.

It would appear that the plasma fibrinogen declines to below normal levels prior to the attack and rises during the attack. Also in one patient, thrombocytopenia has been noted only during the febrile episode.

During the episode the duodenum becomes dilated, the passage of barium is slowed, as is the passage of carmine red dye in certain patients. The absorptive capacity appears to be intact.

One patient studied thus far appears to be more sensitive to small doses of endotoxin, than are normal controls.

Significance to the Program of the Institute:

These studies represent an approach of the clinical investigators mentioned above to better define a poorly understood clinical state that is characterized predominantly by fever and abdominal pain. The study of this particular fever utilizing human sera as a possible pyrogen perhaps can lead to some new insight into the mechanism of fever. The use of immunologic techniques to study this problem may help to define some of the basic etiologic factors in FMF.

Proposed Course of Project:

All of the above mentioned studies are in progress and will continue to be investigated. In addition an attempt to delineate what role, if any, pyrogenic steroids (e.g. etiocholanolone) may be playing in this disease are to be investigated. Genetic studies in collaboration with Dr. B. Blumberg (NIAMD) are anticipated.

Subproject II: Periodic Fever

Objectives:

1. To study a group of patients with recurrent fever, in an attempt to establish what may or may not be the factors common to all in the development of their fever.

2. To characterize the excretion, degradation and secretion of certain "pyrogenic" steroids.

Methods Employed:

Drs. B. Kliman and G. Bryan, NHI, are attempting to develop a technique for measuring certain keto-steroids (androsterone, etiocholanolone, dehydroepiandrosterone) in the gas-chromatograph. All patients undergo thorough clinical and laboratory investigation and in particular response to exogenous corticotrophin, endotoxin and isotropcally labelled "pyrogenic" steroids will be measured.

3. To characterize the immunologic phenomena of FMF.

4. To assess the response of FMF patients to endotoxin.

5. To characterize the plasma fibrinogen changes in FMF (with Dr. Shulman).

6. To confirm if aminoaciduria occurs in FMF.

7. To describe the physiologic and radiologic changes in gastrointestinal function in FMF (with Dr. Laster).

[illegible]

9. To intensively study a group of FMF patients in an attempt to better delineate the clinical syndrome.

Methods Employed:

The procedure for the generalized Schwartzman reaction in rabbits is used, except that sera from patients is substituted as the preparative dose. Temperatures are recorded and the tissue of the rabbits are examined microscopically. The patients' sera are examined by immunoelectrophoresis, and a variety of serologic methods. A standardized preparation of endotoxin (Lipexal$^{(R)}$) is given to the patients and the fever curve, white blood cell count, differential blood counts and fibrinogen measured. The plasma fibrinogen is measured serially during afebrile and febrile periods. Afebrile and febrile urines are chromatographed in the ion-exchange column (method of Stein and Moore) by Dr. L. Levintow's laboratory and the quantitative excretion of amino acids measured. The patient's ability to absorb fats and sugars are measured. Dye markers are used to measure transport time and radiologic changes of the gastrointestinal tract are observed when febrile as well as afebrile. Urinary and plasma steroids when febrile, afebrile and after an infusion of ACTH are measured.

Patient Material:

Patients with familial Mediterranean fever are admitted to the Clinical Center for these studies. Extensive clinical studies including laboratory, radiologic and biopsy studies are done.

Major Findings:

When blood is removed from the patient at the beginning of a clinical attack and infused to a rabbit, preliminary data suggests that it may be pyrogenic. Also when this infusion is followed 24 hours later by an injection of endotoxin, massive central lobular necrosis of the liver, focal necrosis of the kidney and necrosis of the spleen has been seen. On occasion renal cortical necrosis has been noted.

Patient Material:

A small selected group of patients is admitted to the clinical center for these studies.

Major Findings:

Thus far, it would appear that the recurrent or "periodic" fever in these patients is secondary to other disease states. For example, a patient is being studied who probably has a hypothalamic lesion; another has an inability to conjugate etiocholanolone.

[illegible]

The recent demonstration by others that certain steroids may be playing a role in some patients with fever, is a major finding. The availability of these steroids for study may lead to some understanding into the pathogenesis of fever.

Proposed Course of Project:

The above studies will be continued and expanded, as the techniques are developed and improved.

Subproject III: Physiologic Responses in Febrile Patients

Objective:

To determine what effect recurrent fever has upon the responsiveness of patients to environmental temperature changes and exercise.

Methods Employed:

Utilizing the metabolic chamber (NIAMD, Dr. E. Buskirk and R. Thompson) the patients are studied in different environmental temperatures and during controlled exercise tests. The peripheral temperatures, rectal ("core") temperatures, oxygen consumption and CO_2 blow-off are measured continually.

Patient Material:

Three groups of patients comprise the study material: a) the patients with familial Mediterranean fever, b) patients with recurrent fever, c) malaria volunteers (see malaria project).

Major Findings:

Preliminary data suggests that patients who have had recurrent febrile episodes respond metabolically in a different manner to

environmental cold stress than do normal controls. For example a 40 year old patient with FMF does not show the normal elevation of energy expenditure when stressed with cold; and a 14 year old boy with periodic episodes of fever, nausea and vomiting does not respond at all suggesting a hypothalamic lesion.

Significance of Program to the Institute:

This research should lead to a better understanding of some of the physiological changes, if any, that occur in people who have recurrent fevers.

[illegible]

To continue the above program and especially to do the studies before the malaria volunteers become febrile and repeat following cure of their disease.

Part B included: NO

Serial No. NIAID - 12(c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Respiratory Viral Diseases

Principal Investigators: Dr. Vernon Knight
Dr. Julius A. Kasel
[redacted]

[redacted]
Dr. Hugh Evans   Dr. Howard Kravetz 6/30/61
Dr. Robert Couch 9-1-61
Dr. Thomas Cate 7-1-61
Dr. David Lang 7-1-61
[redacted]
Mr. Donald Hollabough
Miss Margaret Huber

Cooperating Units: Dr. Robert Chanock, LID, NIAID
Dr. Karl Johnson, LID, NIAID (Serial No. 72-B)

Man Years (calendar year 1961):
Total: 8.2
Professional: 3.4
Other: 4.8

Project Description:

Objectives:

1. To define clinical entities in relation to newly isolated respiratory viruses.

2. To define diagnostic criteria, pathogenesis, immune response, persistence, sites and effects on tissues of virus in certain infections of respiratory passages and mouth.

3. To study host-parasite relationships in reference to susceptibility to chronic or recurrent respiratory diseases.

4. To improve clinical laboratory techniques in the laboratory confirmation of respiratory viral disease diagnosis.

5. To characterize in man the clinical course and associated virological and immunological phenomena of infection with selected respiratory viral agents.

Methods Employed:

Results of clinical observations and procedures are correlated with bacterial, mycologic and viral isolations employing both animals and tissue culture techniques.

Clinical observations are made under carefully controlled isolation following experimental intranasal infection of normal volunteers with certain viral agents. Virus isolation and immunologic studies performed by recognized procedures.

Patient Material and Major Findings:

[illegible] consisted of a series of studies of induced infection with respiratory agents in volunteers. In addition, a viral infection diagnostic service was offered to selected cases.

The following respiratory agents were studied in volunteers in calendar year 1961:

Agent	No. of Volunteers
Eaton agent of primary atypical pneumonia (46 were studied previously)	6
Influenza A_2 (Asian)	15
Adenovirus 7	24
Adenovirus 16	3
Adenovirus 26	45
Adenovirus 27	15
Coxsackie A-21 (Coe)	36
Coxsackie A-24 (Pett)	9
Enterovirus, unclassified	9
Reovirus, Types 1, 2, and 3	27
	189

The volunteers were inmates of Federal Correctional Institutions. They were brought here by the Normal Volunteer Committee of the Clinical Center, under the direction of Dr. Clifton Himmelsbach and assisted by Mr. Willard Maginnis. Permission for their participation was given by Mr. James Bennett and Dr. Harold Janney, Director and Chief Medical Officer, respectively, of the Bureau of Federal Prisons.

Description of the results of these investigations appear in other project reports of the laboratory and the Laboratory of Infectious Diseases.

The virus diagnostic unit examined 2,456 specimens and obtained a virus from 56 of them.

Significance to Microbiologic Research:

The principle reason for the use of humans as volunteer subjects in studies of respiratory viral agents is that, with minor exception, no animal is susceptible to illness with these agents and naturally-occurring cases of disease are available only in a limited way. The considerable prevalence of most of the agents in the population and the mild nature of illness resulting from infection with them offers considerable assurance of the safety of such tests.

Such studies provide a basis for defining the clinical syndromes of virus infection, their pathogenesis, immune response, effect of size [illegible] provide experimental models by which efficacy of vaccines may be precisely measured. They offer means for controlled studies of factors influencing spread of infection.

Projected Course of the Project:

It appears now that this program will be a continuing one. Ten rooms, with three men assigned to a room, is the present maximum allowance for volunteers. This leave 16 rooms and 32 beds (2 patients per room) for other studies. An increase in volunteers above this limit will jeopardize the continuity of other programs.

A program has been initiated with investigators at Fort Detrick to study aerosol inocula of selected viral agents in volunteers. This has great interest because small particle aerosols can be deposited in peripheral pulmonary bronchioles, a site whose role in pathogenesis of infection has not been well-studied.

Part B included: Yes /X/

Serial No. NIAID -12(c)

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Kravetz, H. M., Knight, V., Chanock, R. M., Morris, J. A., Johnson, K. M., Rifkind, D., and Utz, J. P.: Respiratory syncytial virus: III. Production of illness and clinical observations in adult volunteers. JAMA 176: 657-663, 1961.

2. Johnson, K. M., Chanock, R. M., Rifkind, D., Kravetz, H. M., and Knight, V.: Respiratory syncytial virus: IV. Correlation of virus shedding, serologic response, and illness in adult volunteers. JAMA 176: 663-667, 1961.

3. Chanock, R. M., Rifkind, D., Kravetz, H. M., Knight, V., and Johnson, K. M.: Respiratory disease in volunteers infected with Eaton agent; a preliminary report. Proc. Nat. Acad. Sci. 47: 887-890, 1961.

4. Knight, V., Kapikian, A. Z., Kravetz, H. M., Chanock, R. M., Morris, J. A., Huebner, R. J., Smadel, J. E., and Evans, H. E.: Ecology of a newly recognized common respiratory agent RS-virus. A Combined Clinical Staff Conference at the National Institutes of Health. Ann. Int. Med. 55: 507-524, 1961.

Serial No. NIAID - 13 (c)
1. LCI
2. Pediatric Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Antimicrobial Drug Therapy

Principal Investigator: Dr. Philip Fireman

Other Investigators: Vernon Knight, M.D.
Dr. David Rifkind
Margret Huber

Cooperating Units: None

Man Years (calendar year 1961):

Total:	2.8
Professional:	2.0
Other:	0.8

Project Description:

Objectives:

a) To evaluate an antimicrobic drug.
   1. Effectiveness in treatment of penicillin-resistant staphylococcal infections.
   2. Effectiveness in treatment of other coccal infections.
   3. Appropriate dosages and routes of administration.
   4. Toxic and allergic side effects.
   5. Effectiveness in treatment of the nasal carrier state of staphylococci.

Methods Employed:

Hospitalized and OPD patients with staphylococcal and streptococcal infections were evaluated by clinical and laboratory methods for response to antimicrobics. The laboratory methods included:

a) Isolation and identification of the etiological agent.
b) Determination of the *in vitro* sensitivity of the organism to the drug.
c) Assay of serum and urine for drug concentrations during the course of treatment

Serial No. NIAID - 13 (c)
1. LCI
2. Pediatric Service
3. Bethesda, Maryland

d) Determination of the inhibitory effect of serum against the offending microorganism.
e) Bacteriologic culture of lesions and blood during the course of treatment.

Patient Material and Major Findings:

A. Dimethoxyphenyl penicillin (methicillin)

1. A total of 30 hospitalized patients were treated with dimethoxyphenyl penicillin; 2 with staphylococcal septicemia; 1 with staphylococcal subacute bacterial endocarditis; 1 with staphylococcal arthritis and pneumonia; 2 with chronic staphylococcal osteomyelitis; 4 with staphylococcal pneumonia; 8 with staphylococcal abscesses; 12 with cystic fibrosis of the pancreas; 1 with Group M streptococcal endocarditis and 1 with Group D streptococcal meningitis.

2. All patients treated with dimethoxyphenyl penicillin parenterally showed clinical evidence of improvement or cure. There were no deaths. Therapy was particularly successful in staphylococcal septicemia, abcesses, arthritis, pneumonia and subacute bacterial endocarditis. In patients with cystic fibrosis of the pancreas, staphylococci could be removed from the sputum with parenteral therapy but there has been no change thus far in the nature of the pulmonary disease. Aerosol therapy with dimethoxyphenyl penicillin did not clear staphylococci from the sputum.

3. Daily doses of 8-10 grams I.M. and 10-15 grams I.V. were well tolerated and gave good clinical results. One patient has been treated with 780 grams of the drug over a 130 day period.

4. Probenecid elevated and prolonged blood levels of the experimental penicillin and was used in all patients where it was tolerated.

5. All staphylococci were found to be sensitive to 2-4 mcg/ml of the experimental penicillin; this being true for both penicillin-G-sensitive and resistant strains. This drug was found to be actively bactericidal *in vitro*. Streptococci were also found to be sensitive, however, enterococci were relatively resistant.

6. Blood levels were found to vary from 19.7 to 97.5 mcg/ml at varying times following administration.

7. In 3 cases maculopapular rash resulted from experimental penicillin treatment. In 1 case the rash was severe enough to require discontinuance of the medication.

Serial No. NIAID - 13 (c)
1. LCI
2. Pediatric Service
3. Bethesda, Maryland

B. 5-methyl-3-phenyl-4-isoxazolyl penicillin

1. A total of 12 patients were treated with this new penicillin; one with staphylococcal subacute bacterial endocarditis; one with staphylococcal pneumonia; five with staphylococcal abscesses and 5 with cystic fibrosis of the pancreas.

2. All patients except those with cystic fibrosis of the pancreas showed clinical evidence of improvement or cure. Three cystic fibrosis patients were cleared of staphylococci from their sputum but in the other two patients, there has been no bacteriologic change. Higher dosages of the drug will be evaluated.

3. The drug is acid stable and can be absorbed after oral ingestion. Daily oral doses of 4 to 8 grams were well tolerated in most patients. One patient has received 4 to 6 grams intramuscularly daily. One patient has been treated with 600 grams of the drug over a 150 day period.

4. Probenecid elevated and prolonged blood levels of this new penicillin and was used in most patients.

5. All staphylococci were found to be sensitive to 0.05-0.4 mcg/ml; this was true for penicillin-G sensitive and resistant strains. This new penicillin was shown to be 5 to 10 times more effective in vitro than dimethoxyphenyl penicillin.

6. Blood levels were found to vary from 2.1 to 35.6 mcg./ml following oral administration. Blood levels following I.M. administration were approximately twice those of oral.

7. Two patients experienced nausea and emesis following an oral dose of greater than 2 and 4 grams respectively. No allergic manifestations have been observed. No rash was observed in 3 patients who had a maculopapular rash from dimethoxyphenyl penicillin.

Significance to Biomedical Research:

1. These drugs are of importance, both clinically and theoretically because of their almost total resistance to penicillinase: Its effectiveness in the treatment of penicillin-G-resistant staphylococcal infections is of significance and utility in itself. On a more basic level, it helps clarify the role of penicillinase in microbial resistance to penicillin.

2. These drugs hold promise for the long term treatment of chronic staphylococcal disease such as osteomyelitis, recurrent furunculosis, cystic fibrosis of the pancreas and other diseases due to penicillin resistant staphylococci.

3. These drugs may prove to be a useful agent in the study of penicillin allergy.

4. These drugs, because of their rather narrow microbial spectrum, should provide information on the role of microorganisms in the chronic pulmonary disease associated with cystic fibrosis of the pancreas.

Proposed Course of Project:

1. Continue present clinical studies with particular emphasis on chronic staphylococcal infections and on cystic fibrosis of the pancreas.

2. Extend preliminary observations of allergic reactions to this new penicillin and other drugs and define the extent of cross reactivity with penicillin G.

Part B included: Yes

Serial No. NIAID - 13 (c)
1. LCI
2. Pediatric Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project

1. Knight, V., Bunn, P.A., Steinman, H.G., and Fireman, P.: A new penicillin derivative resistant to penicillinase, dimethoxyphenyl penicillin. A Combined Clinical Staff Conference at the National Institutes of Health. Antibiotics & Chemotherapy 11: 537-554, 1961.

2. Rifkind, D. and Knight, V.: Treatment of staphylococcal and streptococcal infections with dimethoxyphenyl penicillin. In A Symposium on the New Laboratory and Clinical Experiences with Particular Reference to Resistant Staphylococcal Diseases held Sept. 7, 1960, at the State Univ. of N.Y., Upstate Medical Center, Syracuse, N.Y., Paul A. Bunn (Ed.) Syracuse Univ. Press, 1961, pp. 160-171.

Serial No. NIAID - 14(c)
1. LCI
2. Bacteriology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Staphylococcal Disease

Principal Investigator: Miss Rose Lieberman

Other Investigators: Mrs. Jocelyn Blakely
Mr. William Humphrey

Temporarily discontinued due to demands of more pressing project (see NIAID - 17(c)).

Serial No. NIAID - 15(c)
1. LCI
2. Infectious and Pediatric
Disease Services
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Hepatitis and Mononucleosis

This project has been temporarily inactivated due to lack of patient material. If patients become available, the project will be reactivated.

Serial No. NIAID - 16(c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Aseptic Meningitis

This project has been temporarily inactivated due to lack of material. If patients become available the project will be reactivated.

Serial No. NIAID - 17(c)
1. LCI
2. Bacteriology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Allergy-Immunology

Sub-project I: Diseases of Immune Etiology

Principal Investigator: Dr. Howard Goodman

Other Investigators: Dr. Robert Carpenter
Dr. John P. Nasou (to May 1961)
Mr. John Bozicevich (to April 1961)
Dr. Burton Andersen (from July 1961)
Mr. Robert Bowser
Mr. Stanley Ward

Cooperating Units: This project is being continued on a cooperative basis with LCI by the Laboratory of Immunology, NIAID.

Man Years (calendar year 1961):
Total: 4.6
Professional: 2.8
Other: 1.8

Project Description: See NIAID - 145, 145A, and 156.

Sub-project II: Studies of Antibody Production in Normal Mice and Mice with Neoplasms

Principal Investigator: Miss Rose Lieberman

Other Investigators: Mr. William Humphrey
Mrs. Jocelyn Blakely

Man Years (calendar year 1961):
Total: 1.4
Professional: 0.4
Other: 1.0

Project Description:

Objectives:

1. Studies of primary and secondary immune responses of inbred

mice before, during and after the induction of plasma cell ascites tumors by paraffin-oil adjuvant.

2. Similar studies with mice that have been transplanted with specific characterized plasma cell ascites tumors, producing either alpha, beta or gamma globulins.

Methods Employed:

Balb/c mice with ascites induced by intraperitoneal injections of incomplete Freund's adjuvant receive three different treatments. One group is immunized just prior to plasma cell tumor formation (determined by previous studies to be in the vicinity of 5-1/2 months), second group is immunized in the early development of plasma cell tumors, third group is immunized in the late stages of the development of plasma cell tumors. Studies of humoral and cell bound antibody production utilizing hemolysin titrations, quantitative precipitin tests, passive cutaneous anaphylaxis tests, hemagglutination, gel diffusion methods, electrophoresis, ultracentrifugation, isotope tagging and fluorescent techniques are done to measure and characterize the antibodies produced. In addition, the tumor cells from these hyperimmunized mice are transferred into both untreated mice and into tissue culture to determine if passive transfer of production of antibody is possible and also to determine what cells are implicated in the production of antibody.

Major Findings:

Hemolysin titers obtained in the primary response of mice with plasma cell tumors are markedly depressed and appear to be dependent both on the stage of development of the tumor and on the type of globulins produced by the growth of the tumor. The secondary hemolysin titers approach those obtained in mice without tumors in spite of the continued development of the tumors.

Significance to Microbiological Research:

Although the depression of antibody production in the advanced states of neoplastic diseases is well-known, this apparently is not true for the secondary response. Since experimental evidence implicates the plasma cell in antibody production, the differences in the primary and secondary antibody response of animals with abnormal plasma cells needs further elucidation.

Proposed Course of Project:

To continue as outlined under objectives.

Sub-project III: Interactions of Antibodies

Principal Investigator: Miss Rose Lieberman

Other Investigators: None

Man Years (calendar year 1961):
Total: 0.2
Professional: 0.2
Other: -

Project Description:

Objectives:

To study the effect of mixtures of antibodies in conferring passive protection.

Methods Employed:

Mixtures of Salmonella and of Proteus immune rabbit sera are separately investigated to determine the effect of mouse protection of increasing, decreasing or removing specific antibody components present in the antisera combinations.

Major Findings:

Antibodies employed in combination are either simply additive, enhancing or antagonistic in conferring passive protection in mice.

Significance to Microbiological Research:

Antibodies in combination behave differently from the equivalent amounts of the same antibodies used separately and may be more or less effective in conferring protection.

Proposed Course of Project:

To continue as outlined under objectives.

Sub-project IV: Dynamics of Antibody Production

Principal Investigator: Miss Rose Lieberman

Other Investigators: Mr. William Humphrey
Mrs. Jocelyn Blakely

Man Years (calendar year 1961):
Total: 1.4
Professional: 0.4
Other: 1.0

Serial No. NIAID - 17(c)

Project Description:

Objectives:

1. Study of the dynamics of antibody production in ascitic fluid of individual mice.

2. Studies of antibody titer in ascitic fluid of immunized inbred mice of different strains following primary and secondary immunization.

Methods Employed & Major Findings:

The incidence of ascites and the amount of ascitic fluid produced in 17 different strains of mice were recently reported by this laboratory. The information derived from these investigations permit the selection of mouse strains where dynamics of antibody production utilizing purified antigens may be studied. Mice with ascites induced by paraffin-oil adjuvants may be immunized with antigens of high specificity and the total amount of antibody as well as its course of production can be determined in individual mice employing quantitative procedures for measuring antibody.

Significance to Microbiological Research:

Dynamics of antibody production in the mouse is unknown. In order to assess the effect of various types of neoplasms on immune processes and also to determine the course of antibody production in the normal mouse these studies must be done.

Part B included: Yes /X/

Serial No. NIAID - 17(c)

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

1. Lieberman, R., Mantel, N., and Humphrey, W., Jr.: Ascites Production in 17 Mouse Strains. Proc. Soc. Exper. Biol. and Med. 107: 163-165, 1961.

Serial No. NIAID 18(c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Enteric Diseases

Principal Investigator: Dr. Bayard S. Tynes

Other Investigators: Dr. John P. Utz

Cooperating Unit: None

Man Years (Calendar year 1961):

Total: 0.3
Professional: 0.3
Other:

Project Description:

Objectives:

Basic studies of the etiology, pathogenesis and immunology of the Salmonella carrier state:

1. An evaluation of antibiotic and/or surgical therapy.

2. An evaluation of therapy failures with regard to possible gallbladder and hepatic stones, protoplast formation by the Salmonellae, avascular scars in the liver and host response. Clinical studies include the evaluation of a new semisynthetic penicillin, antibiotic levels in the bile, and biopsy specimens of the liver for culture.

Methods Employed:

1. Cultures of the feces, duodenal aspirate, urine, sputum and biopsy material.

2. Antibiotic sensitivity studies.

3. Cholecystectomy.

4. Liver Biopsy for routine and L-form culture, and histology.

5. Immunologic survey.

6. Immunofluorescent studies of all surgical material from these patients.

7. *In vitro* studies utilizing the stones of carriers removed at cholecystectomy.

Patient Material and Major Findings:

Studies thus far indicate that carriers without gallstones may be cured by antibiotics alone; those with stones may be cured by cholecystectomy alone in 70% and by cholecystectomy and antibiotics combined in 90-100%. *In vitro* studies show the infected gallstone impermiable to antibiotics. Treatment of 26 Salmonella carriers has been reported. These studies were presented at the Regional Meeting of the Am. Coll. of Phys. November 18, 1961, and have been submitted to the ACP meeting in Philadelphia.

Significance to Microbiological Research:

Each state in this area lists over 100 typhoid carriers. The spread of typhoid is primarily from carriers. Over 300 cases of Salmonellosis were attributed to our 26 carriers. An evaluation of therapy is needed.

Proposed Course of Project:

To continue as outlined under Objectives.

Part B Included: No

Serial No. NIAID - 19(c)
1. LCI
2. Parasitic Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Clinical Investigations on Helminthic Diseases

Principal Investigator: Henry K. Beye

This project has been discontinued due to transfer of principal investigator. (See Sub-project A, Serial No. NIAID - 20(c).)

Serial No. NIAID 20 (c)
1. LCI
2. Parasitic Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Clinical Investigations in Protozoan Infections and Diseases:

Subproject A - Investigations on Parasitic Infections & Diseases of Man

Principal Investigator: Dr. H. K. Beye

Other Investigators: Dr. S. F. Kuvin, Dr. Robert Coatney, Dr. George Burton, Dr. B. B. G. Nehaul, Dr. L. C. Mootoo, Dr. John Tobie, Dr. Steven Schenker, Dr. Morton Getz and Joan Gurian, Elizabeth Guinn and Deaner F. Lawless.

Cooperating Units : LCI - NIAID
LPC - NIAID
U. S. Administration for International Development (AID), Georgetown, British Guiana Pan American Health Organization

Man Years (Calendar year 1961)
Total: 3.6
Professional: 2.1
Other: 1.5

Project Description:

The activities described are those of interest to the principal investigator before transferring to MARU in April, 1961, and in which there was a "carry over" after his association with MARU. This project report is thus a record of a transitional period, representing a termination of old activities and the beginning of new ones in these areas of research interest.

Objectives:

For Parasitic Diseases:

1. To develop improved diagnostic techniques;
2. To understand host-parasite relations;
3. To describe more accurately disease associated with protozoa and helminths;

4. To elucidate the epidemiology of these infections and diseases.

Methods Employed:

1. Patient procurement through: a) physician referrals; b) screening of U. S. State Department employees and other suitable groups; and c) study of inmate volunteers.

2. Routine laboratory and specialized techniques.

3. Consultant and directive services to countries such as British Guiana.

Major Findings:

Filariasis:

1. Microfilariae of *Wuchereria bancrofti* in heparinized blood remain viable for as long as 23 months when stored at -22°C.

2. Filariasis is a major health problem in British Guiana. Much has already been learned of its epidemiology within the past year. Suggestive evidence indicates that chymotrypsin may be partially effective in reducing the lymphoedema of elephantiasis, although these results will not be evaluated until later this calendar year.

Malaria:

1. In a continuation of the study of human volunteers infected with *Plasmodium cynomolgi bastianellii* reported last year, much new basic clinical pathological data on these infections in man, as well as infections with *P. vivax* has been documented.

Amebiasis:

1. Through an analysis of our experience with more than 5000 State Department employees, epidemiologic and clinical data concerning infection with *Entamoeba* histolytica and resulting disease among this population group has been made available.

Significance to Bio-medical Research and the Program of the Institute:

The results of the research in filariasis and amebiasis contribute to our understanding of these common infections among the world population, while the observations on the viability of microfilariae is important to workers in biochemistry, physiology and parasitology.

The recent demonstration of the possibility of simian malaria contributing toward human disease makes these studies especially significant. In addition, new information has been gained on the human host responses

to vivax malaria. The assessment of the significance of these findings must await future study.

Proposed Course of the Project:

These specific interests will undoubtedly take different directions and emphasis as they are adapted to the capabilities and facilities at MARU. At the same time, work in this area will undoubtedly continue in LCI and other laboratories in Bethesda.

(See following subproject)

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Beye, Henry K.: Filariasis. Cyclopedia of Medicine, Vol. X, pp. 340-344E, 1961.
2. Beye, Henry K., Getz, Morton E., Coatney, G. Robert, Elder, Harvey A., and Eyles, Don E.: Simian malaria in man. American Journal of Tropical Medicine and Hygiene, Vol. 10, No. 3, pp. 311-316, May 1961.
3. Beye, Henry K.: Helminthic diseases and anthelminthic drugs. Clinical Proceedings of the Childrens Hospital, 1961.
4. Beye, Henry K., and Lawless, Deaner.: Viability of microfilariae of Wuchereria bancrofti when stored at -20°C. Journal of Experimental Parasitology, in press or published Fall of 1961.

Honors and Awards relating to this project:

1. Kuvin, S. F., Beye, H. K., Stohlman, F., Jr., Contacos, P. G., Coatney, G. R.: Clinical manifestations and physiological responses in Sporozoite-induced infections in human volunteers.: Presented to American Society of Tropical Medicine and Hygiene, November, 1961.
2. Beye, H. K., and Tobie, John.: Fluorescence of tetracyclines in filarial worms and other parasites.: Presented to the Pacific Science Congress, Honolulu, August, 1961.
3. Beye, Henry K., and Gurian, Joan. The epidemiology and control of filariasis (Wuchereria bancrofti and Brugia malayi) working paper for W.H.O. Expert Committee on Filariasis, Geneva, July, 1961.

Part A:

Subproject B: Clinical and Physiological Investigations in Normal Volunteers Infected with Simian and Human Malarias.

Principal Investigators: Sanford F. Kuvin, John E. Tobie, G. Robert Coatney and Henry K. Beye

Other Investigators: Deaner Lawless, Charles B. Evans and Elizabeth Guinn

Cooperating Units:
Laboratory of Immunology, NIAID
Laboratory of Parasitic Chemotherapy, NIAID (Coatney and Contacos)
Laboratory of Experimental Pathology, NIAMD (F. Stohlman)
Epidemiology and Biometry Branch, NIAMD (B. Blumberg)
Federal Bureau of Prisons

Man Years (See Subproject A)

Project Description:

Since April 1960, a great deal of emphasis was placed on clinical investigations associated with simian malaria. The fundamental objectives in investigating this malaria have remained essentially the same but with greater activities of interest in specific physiological responses. Little emphasis has been given to the investigation of other protozoan or parasitic diseases.

Objectives:

1. Study the clinical manifestations and pathological processes of simian malaria in man, and contrast them with vivax infections.

2. Better understand host parasite relationships as related to malaria.

3. Demonstrate the course of antibody production in malaria utilizing newer serological techniques.

4. Investigate antigenic relationships between the various species of human and simian malaria.

5. Correlate altered protein patterns and antibody response in malaria.

6. Investigate hematological and protein abnormalities in malaria.

7. Determine the environmental relationship to certain genetically controlled diseases (haptoglobinemias and glucose-6-phosphodehydrogenase deficiencies).

Methods Employed.

Methods of patient procurement utilized in 1961 consisted in the use of selected inmate volunteers from the Malaria Project at the Federal Penitentiary in Atlanta, Georgia for study at the Clinical Center. Methods for investigation and study of these research candidates remain the same, that is, close communication and liason with the laboratories and workers participating in these investigations.

I. Malaria Studies in Normal Volunteers - Clinical Manifestations and Physiological Responses of Simian Malaria in Man

Patient Material and Major Findings:

Five inmate volunteers were experimentally infected by sporozoite inoculation with *Plasmodium cynomolgi bastianellii.* The course of their infection was contrasted with two volunteers infected with the Venezualen strain of *P. vivax*. These studies have revealed the following: The group of patients infected with *P. c. bastianellii* demonstrated a prepatent period of 9-15 days, an incubation period of 10-17 days, with the duration of their febrile illness lasting 8-12 days. The maximum parasite density in this group of patients was 1100 parasites/cmm. The patients infected with *P. vivax* demonstrated a prepatent period of 14 days, with an incubation period of 15 days. The duration of their febrile illness was 19 days, with the maximum parasite density controlled to 16,770 parasites/cmm. Both groups of patients exhibited tertian fever patterns, but with the fever rising higher and lasting longer in the *P. vivax* infected patients. Headache, abdominal pain, and hepatosplenomegaly were prominent in the *P. c. bastianellii* infections. Generalized malaise, anorexia, weight loss, and shaking chills were pronounced in the *P. vivax* infected volunteers. Significant laboratory findings in both groups of patients were anemia, leukopenia, thrombocytopenia, an elevated erythrocyte sedimentation rate, and shortened survival time of chromium^{51} labeled red blood cells. The group infected with *P. vivax* demonstrated a moderately severe anemia with a depressed reticulocyte response in comparison to the group infected with *P. c. bastianellii.* Other positive findings were hypoalbuminemia and hypergammaglobulinemia in both infections, and a fall in the excretion of 17-hydroxycorticosteroid hormone in three of the five volunteers infected with *P. c. bastianellii.*

In general, the illness produced in man by this strain of monkey malaria appears less severe than that produced by vivax malaria, although its severity is out of proportion to the numbers of parasites found in the blood.

II. Antibody Production in Human Malaria as Determined by the Fluorescent Antibody Technique.

Patient Material and Major Findings:

No reliable serological test has been available in the past to follow the course of antibody production in malarial infections. The indirect method of immunofluorescence was utilized in this investigation to study antibody response to sporozoite-induced *P. vivax* infections in normal human volunteers.

Blood films containing *P. vivax* were used as the antigen, dehemoglobinized in 0.1% HCl, and rinsed in distilled water and saline. The serum tested for antibody was applied to the slide, allowed to react, and the slide washed in saline. Fluorescein isothiocyanate conjugated horse anti-human globulin was applied, the slide washed in saline, and the preparation examined with fluorescence microscopy.

Malaria antibody was demonstrated about three weeks after infection, and has persisted thus far for 135 days. Antibody production closely followed the appearance of parasites in the blood and high antibody titers persisted for about 65 days after infection, when they gradually began to fall.

These results suggest that this method of serologic testing is specific and provides a sensitive means of titrating antibody produced in malarial infections.

III. The Relationship Between Malaria Antibody Production as Determined by the Fluorescent Antibody Technique to Serum Gamma Globulin Concentrations in Normal Volunteers Infected by Sporozoite Innoculation with P. vivax and the B Strain of P. cynomolgi.

Patient Material and Major Findings:

Four inmate volunteers were experimentally infected by sporozite inoculation with the Chesson and Venezualen strains of *P. vivax*, and five inmate volunteers similarly infected with the B strain of *P. cynomolgi.* The course of malaria antibody was followed using the indirect fluorescent antibody technique, and the production of gamma globulin determined by electrophoretic analysis of their serum proteins. The results of these studies have revealed the following observations. The volunteers infected with *P. vivax* first demonstrated antibody between the 15th and the 23rd day after infection. Maximum antibody titers of 1:320 and 1:5120 were reached 35 to 42 days after infection, and malaria antibody has persisted in their sera for as long as 119 days. Four of the five patients infected with the B strain of *P. cynomolgi* first demonstrated malaria antibody in their sera 24 days after infection. Maximum antibody titers of 1:320 to 1:5120 were reached 31 to 48 days after infection, and malaria antibody has been demonstrated in their sera for as long

as 311 days. All of the infected volunteers demonstrated an increase in their gamma globulin levels after infection. The maximum increase in gamma globulin varied between 0.40 to 0.88 gms per cent. The maximum antibody titer tended to occur at the same time as the maximum gamma globulin rise with a median difference of two days.

These results suggest that the raised gamma globulin levels found in malaria infections correlate with the production of specific malaria antibody as determined by the fluorescent antibody technique, and that the humoral element of malaria immunity is part of the excess gamma globulin produced. Preliminary studies suggest that there is some form of group reaction with regard to the antigenicity of P. vivax and the B strain of P. cynomolgi.

IV. Haptoglobin and Hematological Studies in Malaria. Patient Material and Major Findings.

Haptoglobins are a group of mucoproteins belonging to the alpha-2 proteins and characterized by their affinity for hemoglobin. Human populations can be divided into genetic haptoglobin types termed 1-1, 2-2, 2-1 dependent on their haptoglobin patterns. Among the various phenotypes found, one is located in certain African populations where low or no serum haptoglobin can be demonstrated. The difference in distribution of different phenotypes may be genetically determined, but may be in part due to environmental forces, such as malaria. Reports have been published of low haptoglobin levels in hemolytic anemias, and we have studied the haptoglobin level and life span of red blood cells infected with the B strain of Plasmodium cynomolgi and Plasmodium vivax in normal volunteers in an effort to further describe the pathogenesis of the different phenotypic haptoglobin types. The results of these investigations are as follows. Chromium^{51} survival studies in the five patients infected with the B strain of P. cynomolgi demonstrated a shortened half time of 15 to 18 days, while in the two vivax infected volunteers the half time was 4 days. These findings suggest that the primary factor responsible for the anemia in these diseases is hemolytic. Concomitent with the fall in hemoglobin, there was a fall in haptoglobin up to 100 mgm per cent. During the period of clinical recovery from malaria, an increase in serum haptoglobin levels toward normal took place. These findings suggest that the hemolytic anemia associated with malaria may be responsible for some of the phenotypes found in certain population groups where malaria is endemic.

Significance to Bio-Medical Research:

New reservoirs of human malaria have definitely been elucidated which may have great significance in terms of world-wide malaria eradication. These investigations have served to further characterize the pathophysiological responses of simian malaria in man, and to more adequately understand the pathogenesis of malaria, including other human forms. This and other simian malarias will provide a useful tool in other studies in malaria.

The fluorescent antibody technique has been successfully used to follow the course of antibody production in normal volunteers infected with human and simian strains of malaria. This method has shown itself to be specific and sensitive in studying antibody production in malaria and should prove of considerable value in epidemiological studies and eradication programs. Our present investigations suggest that the raised gamma globulin levels found in malarial infections correlate with the production of malaria antibody, and that the humoral element of malaria immunity is a part of the excess gamma globulin formed. The haptoglobin abnormalities demonstrated in malaria have correlated with the hemolytic anemia produced, and have demonstrated the environmental effect of malaria on this genetic polymorphism.

Proposed Course of Project:

It is anticipated that additional investigations involving the use of inmate volunteers infected with human and simian malarias will be carried out at the Clinical Center. Studies of cross immunity between the simian malarias and the human malarias are programed. The course of antibody production in various simian and human malarias will be followed and correlated with the associated changes in serum protein patterns. The relationship of antibody titer to various types of antimalarial chemotherapy and to the height of parasitemia will also be under investigation. The environmental effects of malaria on haptoglobin phenotypes will continue to be investigated, and it is anticipated that studies relating malaria immunity to sickle cell disease and glucose-6-phosphodehydrogenase deficiency will be undertaken. The project in the clinical investigation of simian malaria in man is one aspect of an intensive program of investigating the role of simian malaria in the epidemiology of human malarias coordinated through the Laboratory of Parasitic Chemotherapy.

Part B included Yes

Serial No. NIAID - 20(c)

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

1. Kuvin, Sanford F., Tobie, John E., Evans, Charles B., Coatney, G. Robert, and Cantacos Peter G.: Antibody production in human malaria as determined by the fluorescent antibody technique. Science: In press.

Serial No. NIAID - 21(c)
1. LCI
2. Pediatric Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Cystic Fibrosis of the Pancreas

Principal Investigator: Dr. Phillip Fireman

Other Investigators: Dr. George T. Bryan (to 6/30/61)
Dr. Richard C. Adler (from 7/1/61)
Dr. David J. Lang (from 7/1/61)

Cooperating Units: None

Man Years (calendar year 1961):
Total: 1.5
Professional: 1.5
Other: - -

Project Description:

Objectives:

1. To study the microbial flora in patients with cystic fibrosis of the pancreas.

2. To evaluate the role of staphylococcal immunology.

3. To evaluate the effect of certain antibiotic agents on the bacterial flora of the respiratory tract.

Patient Material:

Children with proven cystic fibrosis of the pancreas who are being followed regularly in this clinic.

Major Findings:

The major findings are limited to the study of the microbial flora in patients with cystic fibrosis of the pancreas. These would indicate that children with this disease have a relatively stable bacterial flora in spite of continuous therapeutic antibiotic therapy.

Proposed Course of Study:

This program will be discontinued as opportunity permits.

Part B included: No/X/

Serial No. NIAID - 22(c)
1. LCI
2. Biochemistry & Immunochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Basic Biochemical Studies

Principal Investigator: Dr. Harry G. Steinman

Other Investigators: Dr. Michael Brandriss
Miss Emmy Lou Denny

Man Years (Calendar Year 1961)
Total: 2.8
Professional: 1.0
Other: 1.8

Project Description:

Objectives:

The original program on the biochemistry of pathogenic microorganisms has developed in two directions. The first aspect deals with penicillinase both as the basis for the natural resistance of certain strains of pathogenic staphylococci and as an inducible enzyme. The second aspect deals with examining the manifold reactivities of penicillin ranging from microbiologic to pharmacologic and correlating them with chemical structure.

Methods Employed:

I. Penicillinase

1) Nature of penicillin resistance

The role of penicillinase in the resistance of *Staphylococcus aureus* is being investigated under a variety of conditions.

2) Induced enzyme formation

Penicillinase-producing staphylococci are stimulated to form increased amounts of enzyme. The mechanism of this reaction is under investigation.

3) Penicillinase as a protein

Penicillinase in *S. aureus* is an intracellular enzyme. The characteristics of this protein are being compared with the corresponding enzyme from *Bacillus cereus* where it is largely excreted as an extracellular enzyme.

II. Penicillin

The following biologic activities are being determined with a series of penicillins with different chemical structures in order to determine the effect of chemical modifications:

1) Microbiologic Activities
   a. Antibiotic Activity
   b. Cell-wall Binding

2) Biochemical Activities
   a. Induced Enzyme Formation
   b. Reactivity to Penicillinase
   c. Stability to Acid

3) Pharmacologic Activities
   a. Serum Protein Binding
   b. Immunologic Responses

Major Findings:

The isolation of cell-free staphylococcal penicillinase in this Laboratory has been instrumental in the rapid evaluation of the many new penicillins now available as the result of the isolation of 6-aminopenicillanic acid, the precursor of penicillin. These findings have been described in a number of papers which have appeared in the past year.

Some of the factors governing the induced formation of penicillinase by staphylococci have been described in a published report.

Significance to Medical Research:

The emergence of 2,6-dimethoxyphenyl penicillin as the standard form of therapy at this time for penicillin-resistant staphylococcal infections reflects the validity of the microbiologic and biochemical data obtained in our laboratory. Some of our findings were among the first to be published on this new penicillin. Similar pioneer studies have been made on methyl-phenyl-isoxazolyl penicillin, a penicillin not yet cleared by the FDA but which promises to be of great usefulness as a penicillinase-resistant penicillin which is effective when taken orally, and on -amino benzylpenicillin, another new type of penicillin with antibiotic activities against gram-negative organisms as well as gram-positive organisms. The integration of the findings from the

basic laboratory studies and from the clinical investigations have established this Laboratory as an important center in the evaluation of the new penicillins.

Proposed Course of Project:

Continuation of this integrated effort together with cooperative studies with the Laboratory of Immunology promises to furnish fundamental concepts in the mechanism of penicillin action in man, where it is exposed to a variety of competitive forces and where, although non-toxic *per se*, it occasionally acts as an allergenic haptene with severe consequences.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1960

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Steinman, H. G.: Factors modifying induced formation of penicillinase in *Staphylococcus aureus.* J. Bact. 81: 895-902, 1961.

2. Steinman, H. G.: A biochemical comparison of 6-aminopenicillanic acid, benzylpenicillin, and 2,6-dimethoxyphenylpenicillin. Proc. Soc. Exp. Biol. Med. 106: 227-231, 1961.

3. Steinman, H. G.: Biochemical studies on 6-amino-penicillanic acid, benzyl penicillin, and 2,6-dimethoxyphenyl penicillin. In A Symposium on the New Dimethoxyphenyl Penicillin - Early Laboratory and Clinical Experiences with Particular Reference to Resistant Staphylococcal Diseases held Sept. 7, 1960, at the State Univ. of N. Y., Paul A. Bunn (Ed.), Syracuse Univ. Press, 1961, pp. 17-25.

4. Steinman, H. G.: Preparation of cell-free extracts of staphylococcal penicillinase. J. Bact., V82, pp. 620-621, 1961.

5. Steinman, H. G.: A comparison of the biochemical activities of 6-aminopenicillanic acid, benzylpenicillin, 2,6-dimethoxyphenyl penicillin, -phenoxyethylpenicillin, and phenoxymethyl penicillin. In Gray, P. (Ed.): Antimicrobial Agents Annual-1960. New York, Plenum Press 1961, pp. 256-263.

6. Knight, V., Bunn, P. A., Steinman, H. G., and Fireman, P.: A new penicillin derivative resistant to penicillinase, dimethoxyphenyl penicillin. A Combined Clinical Staff Conference of the National Institutes of Health. Antibiotics & Chemotherapy 11: 537-554, 1961.

Honors and Awards relating to this project:

1. Steinman, H. G.: The biochemical basis for the efficacy of 2,6-dimethoxyphenyl penicillin against penicillin-G-resistant *Staphylococcus aureus.* Presented at 42nd Annual Session of American College of Physicians, May 8-12, 1961, Miami, Florida, by invitation.

Serial No. NIAID-23(c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Systemic fungal disease

Principal Investigator: Dr. John P. Utz

Other Investigators: Dr. Bayard S. Tynes, Dr. John E. Bennett, Dr. Michael Brandriss, Dr. William Butler, Dr. George Hill, Dr. Howard Kravetz, Dr. Vincent Andriole and Margret Huber.

Cooperating Units: Laboratory of Infectious Diseases, NIAID-76-B.

Man Years (calendar year 1961):
Total: 3.5
Professional: 3.3
Other: 0.2

Project Description:

Objectives:

1. To study diagnosis, pathogenesis and natural course of systemic fungus infections.

2. To study the problem of fungus disease due to overgrowth during antibiotic and steroid treatment.

3. To evaluate new agents in antibiotic and chemotherapeutic treatment of systemic fungus infections.

4. To set up means of measuring blood levels of new antifungal agents and to study acute and long term toxic effects of the drugs.

5. To study various immunologic aspects of this group of diseases.

Methods Employed:

Patients with suspected fungal infections are studied diagnostically using the skin test, serologic, histologic, and cultural methods. Family contacts are similarly investigated and environmental studies undertaken, when indicated. Infecting fungi from such patients are screened for sensitivity against a considerable number of chemothera-

peutic or antibiotic agents and therapy is undertaken after such studies and where clinically advisable.

Drugs are screened for activity against an organism isolated from a patient by in vitro methods. Promising drugs are then tested in animals for acute and long-term toxicity and for therapeutic effectiveness.

New methods are being developed for determining serum drug levels, rates of excretion of drug, and in vitro screening of drugs.

Patient Material and Major Findings:

1. New patients admitted for study this year include those with histoplasmosis (8), cryptococcosis (12), blastomycosis (6), sporotrichosis (1) and phycomycosis (1).

2. The studies of renal function in patients before, during and after amphotericin have been concluded and a paper describing this investigation has been accepted for publication.

3. Studies have been instituted to define the mechanisms involved in the serum hypokalemia that occurs generally with amphotericin therapy. The pathology of the kidneys of patients and animals will also be studied more intensively.

4. Radioactive iron and chromium tagged erythrocyte techniques have been used in a study of the anemia that similarly accompanies amphotericin therapy. One or two additional patients are needed to complete this study.

5. The investigation of therapeutic and other effects of the new antibiotic X-5079C (Ro-2-7758) have been published in a series of six articles. The distribution, excretion and fate of this antibiotic are matters of continuing study in additional patients.

6. The extensive review of Candida endocarditis based in part on studies of 6 patients here has been accepted for publication.

7. With the use of the "double blind" technique various drugs including doses of corticosteroids, acetyl salicylic acid, and diphenhydramine are being tested for their ability to lessen amphotericin side effects, serious impediments at present to the successful treatment of the systemic mycoses. Over 400 treatments have been studied thus far. With lower doses of steroid the frequency of reactions was reduced only minimally (from 80% of injections to 60%).

8. The clinical and laboratory aspects of histoplasma meningitis are being analyzed in the 5 adult patients studied here over the past few years. This manifestation is considered either unique or, more likely, rarely recognized since it has been reported previously in only 2 patients (children).

9. The impact of amphotericin B in the treatment of cryptococcal meningitis is being assessed in the 35 cases of disease studied here. Analysis of these patients reveals that the poorest response to therapy occurs in those patients with an underlying disease such as diabetes, Hodgkin's disease or in those patients on corticosteroid treatment. Relapse occurs in approximately 15% of treated cases and oddly does not seem related to total dosage previously administered or to underlying disease such as the above.

10. A study of the oral administration of the present form of amphotericin has been completed and published. Despite the excellent results of such therapy in animals the drug by this route was poorly tolerated in every patient studied. Nausea, vomiting and diarrhea were so severe as to preclude adequate absorption of the antibiotic. In only a few patients were effective blood levels achieved and even then only with 100 times the dosage effective by the intravenous route. A good part of this toxicity should be blamed on the desoxycholate component of the solubilized drug as pure amphotericin is well tolerated orally.

11. Although 2 of the first 3 cases of histoplasmosis reported by Darling had gastrointestinal lesions the role of this common infection in ulcerative disease of the mouth and bowel has been undoubtedly overlooked. The records of patients here with this manifestation of infection are being analyzed in an attempt to uncover characteristic clinical features which might lead to more frequent recognition.

Significance to Microbiological Research:

The group of fungus diseases under study is poorly understood; there is no uniformly successful treatment. Apparently, certain of them, such as histoplasmosis, are being recognized as quite prevalent; others such as candidiasis create problems by the very ubiquity of the organism, which may grow more abundantly and cause systemic disease in the presence of most antibiotics and steroids, creating serious and extensive clinical problems.

Proposed Course of Project:

To proceed as outlined under "Objectives" and "Methods."

Part B included: Yes

Serial No. NIAID - 24(c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Sarcoidosis

Principal Investigator: Dr. Sheldon M. Wolff

Other Investigators: Dr. Thomas R. Cate
Dr. Howard C. Goodman, LI, NIAID

Cooperating Units: None

Man Years (calendar year 1961):
Total: .25
Professional: .25
Other: --

Project Description:

Objectives:

1. To study a small select group of patients with active disease to ascertain the effects of the antimalarial drugs on sarcoidosis.

2. To better define some of the immunologic phenomena that occur in this disease.

Methods Employed:

Patients are subjected to a series of clinical studies according to a protocol. Standard immunologic techniques such as immuno-electrophoresis, serology, antibody titers following antigenic stimuli and others will be employed.

Patient Material:

Most patients are followed in the out-patient clinic of the Clinical Center, but when necessary are admitted to the hospital.

Major Findings:

The project is in an early phase, but thus far it would appear that hydroxychloroquin is having a beneficial effect in a few patients.

Significance to the Program of the Institute:

A small, but well-studied group of patients with this poorly understood disease, may help to clarify some of the clinical and immunologic phenomena that occur. Any advance in therapy will be of significance.

Proposed Course of Project:

Since this project is just now beginning, it will follow the course as outlined above.

Part B included: No /X/

Serial No. NIAID - 25 (c)
1. LCI
2. Infectious and Pediatric Disease Services
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Pyelonephritis

This project has been temporarily inactivated due to lack of investigators. Project to be reactivated at a proper time in the future.

Serial No. NIAID 26 (c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Urinary Tract Viruses

Principal Investigator: Dr. John P. Utz

Other Investigators: C. F. Szwed

Cooperating Units: None

Man Years (calendar year 1961):

Total: 0.6
Professional: 0.6
Other: -

Project Description:

Objectives:

1. To study the urine of all patients with viral infection for the presence of viruses and to study renal function concurrently.

2. To investigate in animals the effect on the kidneys of the inoculation of virus recovered from human urine.

3. To attempt to produce pyelonephritis in animals by virus inoculation alone or accompanied by secondary physiologic trauma or bacterial infection.

Methods Employed:

Clinical Studies: Patients with a variety of viral infections will be studied intensively.

Laboratory Studies: Tissue culture and animal inoculation techniques will be used.

Patient Material and Major Findings:

1. A joint study has been underway with the U. S. National Naval Medical Center studying a group of 24 patients with mumps. Urines have

been cultured for virus, serum for antibody rise, and renal function has been determined. Mumps virus has been recovered from a patient as late as the seventeenth day of illness. Cell culture techniques have proved far superior to the embryonated hen's egg in isolating virus. In cell cultures a cytopathic effect was a more sensitive indicator of virus than was hemadsorption. Hemagglutination was present in 82% of cell cultures positive for hemadsorption.

The clinical data in these patients have not been analyzed as yet.

2. In continuing studies of Coxsackie and ECHO infections in mice virus has been cultured in titers of 10^4 to 10^6 infectious particles per gram of tissue and lesions have been seen in the proximal convoluted tubules. Fluorescent antibody techniques suggest localization of antigenic (viral) material at this latter site. Antibody does not seem, however, to be of sufficient strength to lead to unequivocal conclusions. Studies are continuing in an effort to produce more effective tagged antiserums.

Significance to Microbiological Research:

The recovery of a number of viruses from the urine of patients raises the question of the role they play in disease. The relationship of this infection to subsequent pyelonephritis needs to be explored.

Proposed Course of Project:

As outlined in "Objectives" and "Methods."

Part B included: Yes

Serial No. NIAID - 27(c)
1. LCI
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Biochemical Studies on Staphylococcal Cell Walls

Principal Investigator: Dr. Eskin Huff

This project and investigator transferred to LID, Medical and Physiological Bacteriology Section 1/1/61.

Serial No. NIAID - 28(c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Pathogenesis of Viral Infections

Principal Investigator: Dr. Julius A. Kasel

Other Investigators: Hugh Evans, M. D.

Cooperating Units: None

Man Years (calendar year 1961):

Total:	0.5
Professional:	0.3
Other:	0.2

Project Description: (see instructions)

Objectives:

1. To study the biologic and physiologic significance of antigens produced as a consequence of viral infection in order to assess their role in the pathogenesis of infection.

2. To study the effect of topical and nasopharyngeal administration of adenovirus inhibitor and myxovirus interferon suspensions in volunteers inoculated with different viruses in order to evaluate the modification of clinical illness, viral shedding and immunologic response.

3. To study blood cells from volunteers experimentally infected with a variety of viruses for the presence of viral antigen by fluorescent microscopy concurrently with virus isolation procedures.

4. To determine sialic acid levels of serum and urine from patients ill with respiratory viruses.

Methods Employed:

Clinical studies: Material from human volunteers inoculated with different viruses and patients with non-viral illnesses will be utilized in the projects under study.

Laboratory studies: Tissue culture, chromatography, fluorescent microscopy, immunoelectrophoresis, serologic and biologic techniques will be utilized.

Major Findings:

1. Recent adenovirus type 1 and 2 isolates and prototype strains 1, 2, 4 and 15 were found to possess the property of removing erythrocyte receptor sites possibly by enzymatic action, from human red blood cells for three of five adenovirus hemagglutinins. The antigen responsible for this property is separable from the infectious virus particle and not related to the antigens causing cell detachment and early cytopathogenicity. Studies indicated that the adenovirus factor was a macromolecular substance, heat stable, resistant to proteolytic and nuclease enzymes and was neutralized by homologous but not heterologous serum.

2. Adenovirus Type 1 suspensions devoid of infectious virus and cell detaching factor exhibited an "inhibiting" property for adenovirus type 26 *in vitro*.

Significance to Microbiological Research:

1. The presence of viral antigens other than infectious viral particles in infectious tissue culture fluids raises the question of their significance in disease and immunity. Their role in viral reproduction and possible effect on the human host needs to be defined.

2. Since *in vitro* studies with the adenovirus inhibitor and interferon indicate a suppressive effect upon viral multiplication, the effect of these materials in human viral illnesses needs to be defined.

3. Determination of sialic acid levels in the blood and urine of patients ill with viral infections may warrant this biochemical assay as a clinical tool. The further definition of the enzyme-like factor associated with adenoviruses needs to be further explored for reaction products also, for consequent use as a diagnostic test.

Proposed Course of Project:

As outlined in "Objectives and Methods."

Part B included: Yes

Serial No. NIAID - 28(c)

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Kasel, J. A., Rowe, W. P. and Nemes, J. C.: Further Characterization of the Adenovirus Erythrocyte Receptor-Modifying Factor. J. Expt. Med. 114:717-728, 1961.

Serial No. NIAID - 29(c)
1. LCI
2. Bacteriology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Mid-line Granuloma and Wegener's Granulomatosis

Principal Investigator: Dr. Sheldon M. Wolff

Other Investigators: Dr. Vernon Knight

Cooperating Units: Dr. Kirkland Brace, NCI

Man Years (calendar year 1961):

Total:	.25
Professional:	.25
Other:	-

Project Description:

Objectives:

1. To determine if a recoverable agent plays a role in the etiology of these diseases.

2. To define the clinical state and course of this group of diseases.

3. To attempt a rational approach at therapy.

4. To define what, if any, specific immunologic phenomena occur in these diseases.

Methods Employed:

A variety of standard bacteriologic, mycologic and viral cultures are used in attempt to isolate a specific organism. Extensive clinical and laboratory examinations are made according to a protocol. Standard immunologic techniques of immuno-electrophoresis, fluorescent antibody, gel diffusion and others are planned. Irradiation is given using standard equipment.

Patient Material:

Patients with granulomatous disease of the mid-line facies or of the disseminated type with vasculitis are admitted to the Clinical Center. The patients are thoroughly evaluated with a variety of techniques as

outlined above. When indicated the patients are given a course of radiotherapy and followed subsequently.

Major Findings:

In a small group of patients, extensive studies have failed to reveal a specific micro-organism recoverable from these patients. Although heretofore almost always a uniformly fatal disease (mid-line granuloma), we have preliminary evidence suggesting that high doses of radiation (5000 R) can have marked palliative and/or therapeutic effects. In fact, one patient is now having reconstructive surgery, fifteen months after irradiation of an extensive mutilating lesion.

Significance to Program of the Institute:

Granulomatous disease is usually attributable to infectious agents. However, there remains a group of disease states of unknown etiology that appear clinically to be related to infectious diseases, but await better clarification. Progress in this group should lead to better understanding of other granulomatous diseases and/or possible "hypersensitive states".

Proposed Course of Project:

Surgical and/or autopsy tissue from four patients has been freshly frozen and in collaboration with Dr. Arthur Strauss, LI, immunofluorescent microscopy will be done. Utilizing normal nasal mucosa as antigen an attempt will be made to demonstrate antibody in the patients sera.

Part B included: No /X/

Serial No. NIAID 30(c)
1. LCI
2. Biochemistry and Immuno-Chemistry Section
3 Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Studies on Immediate and Delayed Sensitivity to Simple Chemicals and Proteins

Principal Investigator: Dr. Michael Brandriss

Other Investigators: Dr. Harry G. Steinman

Cooperating Units: Dr. Sanford Stone
Laboratory of Immunology, Serial No.141, NIAID

Man Years (calendar year 1961):

Total:	2.3
Professional:	0.5
Other:	1.8

Project Description:

I. Hypersensitivity to Penicillin and Related Simple Chemicals

Objectives:

Studies on the antigenic determinant(s) of the penicillin molecule responsible for induction of hypersensitivity to simple chemicals. Strain and species differences among animals are also studied.

Methods Employed:

Penicillin, various alterations of the penicillin molecule and related compounds are used to sensitize two strains of histocompatible guinea pigs as well as random bred animals. Sensitivity to the delayed contact type is elicited with the sensitizing compound and related substances. Experiments in passive sensitization and in antibody production are in progress.

Major Findings:

Contact sensitivity was produced in guinea pigs with penicillin-G and with dimethoxyphenyl penicillin. These compounds cross react in this system. With the same method a modified penicillin molecule altered in one position of the thiazidine ring failed to sensitize in

preliminary experiments. Sensitivity was more uniform in inbred than in random bred animals and strain 2 manifested better sensitivity than did strain 13. This is in contrast to tuberculin and ovalbumin sensitivity where strain 13 is better. Passive transfer of delayed contact sensitivity to penicillin with viable cells was accomplished. Cross reacting hemagglutinating antibody was produced in rabbits to penicillin-G and dimethoxyphenyl penicillin.

Significance to the Program of the Institute:

Simple chemicals offer an advantage in the study of hypersensitivit since they are clearly defined antigens and analogues are easily prepared for investigation of specificity. Hypersensitivity to penicillin is also an important clinical problem.

Proposed Course of Project:

Attempts at passive transfer of sensitivity with cell-free extracts are in progress. More test compounds and protein conjugates of these compounds are in preparation. These will be used in experiments on specific stimulation and inhibition of the immune mechanism including antibody production in penicillin sensitivity.

II. The local hemorrhagic reaction in guinea pigs.

Objectives:

To study the mechanisms of the local hemorrhagic reaction, particularly with respect to its relation to the presence of circulating antibody and to delayed type hypersensitivity.

Methods Employed:

Delayed type sensitivity to protein antigens in produced in guinea pigs by specific precipitates in antibody excess. These animals which are highly sensitive as measured by skin tests, but have no detectable antibody, are challenged with subcutaneous injection of large amounts of antigen.

Major Findings:

Animals with delayed type sensitivity in the absence of antibody do not show this type of massive hemorrhage.

Significance to the Program of the Institute:

The local hemorrhagic reaction recently described by Stone appears to be another manifestation of delayed type sensitivity in the experimenta animal and may be particularly useful in demonstrating the presence of

delayed sensitivity when antibody is present. It may also be useful in comparing the sensitivity produced by injection of proteins with mycobacteria to that produced by injection of immune precipitates, a relationship which is not clear at the present time.

Proposed Course of Project:

Passive sensitization with antibody precipitate sensitized animals will be done. The reactions of these animals will be studied.

Part B included: No

LABORATORY OF INFECTIOUS DISEASES

Summary 1

60 - Studies on Cellular and Cell-Free Staphylococcal Penicillinase 37
60-A - Antibiotic Activity of Sea Water 40
61 - The Electron Transport System in an Autotrophic Hydrogenomonad 43
62 - Biochemical Studies on Staphylococcal Cell Walls ... 46
63 - Non-specific Immunity Associated with Serum Siderophilin and the Iron Metabolism of both Pathogen and Host 50
63-A - Biochemistry of the Acquisition of Iron by Mammalian Tissues as Mediated by the Iron-binding Protein, Siderophilin 54
63-B - Coliform and Pseudomonas Urinary Tract Infections Refractory to Antibiotic Drug Therapy 57
64 - Detoxification of Potential Tuberculostatic, Fungistatic, Parasiticidal and Viricidal Agents 58
64-A - Potential Fungistatic, Parasiticidal, Leprocidal and Viricidal Agents 62
64-B - Isolation, Purification and Characterization of Antibacterial and Antiviral Substances from Mollusks 64
65 - The Biology of Group A Streptococci 67
66-A-J Epidemiologic Studies of Illnesses and Microbial Experience of Junior Village Nursery Children 71
66-K - Epidemiological Studies on Efficacy and Safety of Influenza Vaccine in General Population Groups 80
66-L - Epidemiological Studies on Efficacy and Safety of Asian Influenza Vaccine in General Population Groups 1957-1958 82
66-M - Hemagglutination Inhibition (HI) Antibody to Type A and B Influenza 8-9 years after Vaccination with 100 CCA Units of Monovalent Mineral Oil Adjuvant Vaccine 85
66-N - Inoculation of Parainfluenza Virus Type 3 in Adult Volunteers 88
66-O - The Etiology of Respiratory Disease on a College Campus 91
66-P - The Role of Viruses in the Etiology of Ear Disease . 93
67 - Studies on Enteroviruses, Adenoviruses, Reoviruses . 94
67-A - Studies on Viruses of the Enteric Tract of Cattle .. 99
67-B - Studies on Eosinophilic Meningitis in the Pacific Area 102
67-C - A Study of Squamous Cell Carcinoma (Cancer Eye) in Cattle 106
68 - Studies of Tumor Viruses in Nature 108
68-A - Sero-epidemiology of Virus Infections 114
69-A - Study of non-bacterial Respiratory Disease A) Children 117

LABORATORY OF INFECTIOUS DISEASES

Summary 1

60 - Studies on Cellular and Cell-Free Staphylococcal Penicillinase 37
60-A - Antibiotic Activity of Sea Water 40
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64 - Detoxification of Potential Tuberculostatic, Fungistatic, Parasiticidal and Viricidal Agents 58
64-A - Potential Fungistatic, Parasiticidal, Leprocidal and Viricidal Agents 62
64-B - Isolation, Purification and Characterization of Antibacterial and Antiviral Substances from Mollusks 64
65 - The Biology of Group A Streptococci 67
66-A-J Epidemiologic Studies of Illnesses and Microbial Experience of Junior Village Nursery Children 71
66-K - Epidemiological Studies on Efficacy and Safety of Influenza Vaccine in General Population Groups 80
66-L - Epidemiological Studies on Efficacy and Safety of Asian Influenza Vaccine in General Population Groups 1957-1958 82
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67-A - Studies on Viruses of the Enteric Tract of Cattle .. 99
67-B - Studies on Eosinophilic Meningitis in the Pacific Area 102
67-C - A Study of Squamous Cell Carcinoma (Cancer Eye) in Cattle 106
68 - Studies of Tumor Viruses in Nature 108
68-A - Sero-epidemiology of Virus Infections 114
69-A - Study of non-bacterial Respiratory Disease A) Children 117

69-B - Study of Non-Bacterial Respiratory Disease B) Adults: Military Populations 122
70-A - Laboratory Studies of Newly Recognized Viruses Associated with Respiratory Illness A. Eaton Agent, Para Influenza Viruses, and Respiratory Syncytial Virus 126
70-B - Laboratory Studies of Newly Recognized Viruses Associated with Respiratory Illness B) Enteroviruses 130
71 - Studies of Tumor-producing Viruses 133
71-B - Studies of Mouse Viruses with Special Reference to Their Importance as Extraneous Viruses ("Background Noise") in Mouse Tumor Systems 136
72-A - Study of Respiratory Viruses in Human Volunteers A) Enteroviruses and Respiratory Syncytial 141
72-B - Study of Respiratory Viruses in Human Volunteers B) Eaton Agent and Adenoviruses 145
76-A - Ecologic Studies of Fungi Pathogenic to Man 148
76-B - *In vivo* Tests of Antimycotic Drugs and Antibiotics . 151
76-C - Identification and Study of New and Unusual Fungi from Mycoses 154
78-A - Biochemistry and Physiology of Pathogenic Fungi. (*In vitro* Studies of the Action of Antifungal Agent on Pathogenic Fungi) 157
78-B - Studies on the Physiology of *Coccidioides Immitis* .. 160
79-A - Immunity Studies with Pathogenic Fungi 162
79-B - Effect of Physiological Alteration on the Pathogenesis of Yeast Infections 165
79-C - Antigenic Studies on Candida 168
79-D - Studies on Cryptococcus 171
79-E - Studies on the Toxicity of *Candida albincans* 173
79-F - Role of the *Reticulo-Endothelial* System in Immunity Against Pathogenic Fungi 175

LABORATORY OF INFECTIOUS DISEASES

Project No.	Title	Principal Investigator (s)
Summary		
[illegible]	Studies on Cellular and Cell-Free Staphylococcal Penicillinase.	Dr. A. K. Saz
[illegible]-A	Antibiotic Activity of Sea Water	Dr. A. K. Saz
[illegible]	The electron transport system in an autotrophic hydrogenomad.	Dr. R. Repaske
[illegible]	Biochemical studies on staphylococcal cell walls.	Dr. E. Huff
[illegible]	Non-specific immunity associated with serum siderophilin and the iron matabolism of both pathogen and host.	Dr. A. L. Schade
[illegible]-A	Biochemistry of the acquisition of iron by mammalian tissues as mediated by the iron-binding protein, siderophilin.	Dr. A. L. Schade
[illegible]-B	Coliform and pseudomonas Urinary tract infections refractory to antibiotic drug therapy.	Dr. A. L. Schade
[illegible]	Identification of potential tuberculostatic, fungistatic, parasiticidal and viricidal agents.	Dr. B. Prescott
[illegible]-A	Potential fungistatic, parasiticidal, tuberculocidal and viricidal agents.	Dr. B. Prescott
[illegible]-B	Isolation, purification and characterization of [illegible] and antiviral substances from [illegible].	Dr. B. Prescott
[illegible]	The biology of group A streptococci.	Dr. R. M. Cole Dr. J. J. Hahn
66-A-J	Epidemiologic studies of illnesses and microbial experience of Junior Village nursery children.	Dr. A. Z. Kapikian Dr. J. A. Bell
66-K	Epidemiological studies on efficacy and safety of influenza vaccine in general population groups.	Dr. J. A. Bell Dr. R. N. Phillips Dr. D. J. Davis
66-L	Epidemiological studies on efficacy and safety of Asian Influenza vaccine in general population groups 1957-58.	Dr. R. Seltser
[illegible]-M	Hemagglutination inhibition (HI) antibody [illegible] type [illegible] influenza [illegible] - [illegible] years after vaccination with 100 CCA units of monovalent mineral oil adjuvant vaccine.	Dr. F. M. Davenport Dr. A. V. Hennessy Dr. J. A. Bell
[illegible]-N	Inoculation of parainfluenza virus type 3 in adult volunteers	Dr. A. Z. Kapikian
66-O	The etiology of respiratory disease on a college campus.	Dr. A. Z. Kapikian

66-F	The role of viruses in the etiology of ear disease.	Dr. A. Z. Kapikian
67	Studies on enteroviruses, adenoviruses, reoviruses.	Dr. L. Rosen
67-A	Studies on viruses of the enteric tract of cattle.	Dr. L. Rosen Dr. F. R. Abinanti
67-B	Studies on eosinophilic meningitis in the Pacific area.	Dr. L. Rosen
67-C	A study of squamos cell carcinoma (Cancer Eye) in cattle.	Dr. M. Abinanti Dr. W. Gay Dr. M. Stanton
68	Studies of tumor viruses in nature.	Dr. R. J. Huebner Dr. W. P. Rowe
68-A	Sero-epidemiology of virus infections.	Dr. J. L. Sever Dr. R. J. Huebner
69-A	Study of non-bacterial respiratory disease A) Children	Dr. R. M. Chanock
69-B	Study of non-bacterial respiratory disease B) Adults: Military populations.	Dr. K. M. Johnson Dr. R. Chanock
70-A	Laboratory studies of newly recognized viruses associated with respiratory disease. A. Echo 28, para-influenza viruses & respiratory syncytial virus.	Dr. R. M. Chanock
70-B	Laboratory studies of newly recognized viruses associated with respiratory disease. B) Enteroviruses.	Dr. K. M. Johnson
[illegible]	Studies of tumor-producing viruses.	Dr. [illegible] Rowe Dr. J. W. Hartley
[illegible]-B	[illegible] viruses with special reference to their importance as extraneous viruses ("background noise") in mouse tumor systems.	Dr. J. W. Hartley Dr. W. P. Rowe
72-A	Study of respiratory viruses in human volunteers. A) Enteroviruses and respiratory syncytial.	Dr. K. M. Johnson Dr. R. M. Chanock
[illegible]	Study of respiratory viruses in human volunteers. B) Echo 28 and adenoviruses.	Dr. R. M. Chanock Dr. K. M. Johnson
[illegible]	Experimental studies of fungi pathogenic to man.	Dr. C. W. Emmons
[illegible]	*In vitro* tests of antifungal drugs and antibiotics.	Dr. C. W. Emmons
76-C	Identification and study of new and unusual fungi from mycoses.	Dr. C. W. Emmons
78-A	Biochemistry and Phisiology of pathogenic fungi. (*In vitro* studies of the action of antifungal agent on pathogenic fungi).	Dr. G. W. Lones

78-B	Studies on the physiology of Coccidioides immitis.	Dr. G. W. Lones
79-A	Immunity studies with pathogenic fungi	Dr. H. F. Hasenclever
79-B	Effect of [illegible] alteration on the pathogenesis of yeast infection.	Dr. H. F. Hasenclever
79-C	Antigenic studies on Candida.	Dr. H. F. Hasenclever
79-D	Antigenic Studies on Cryptococcus.	Dr. J. Bennett
79-E	Studies on the toxicity of Candida albicans.	Dr. H. F. Hasenclever
79-F	Role of the reticulo-endothelial system in immunity against pathogenic fungi.	Dr. D. F. Summers

animal to lab use. The Oncogenic Virus Unit is occupying Room 26 as a sterile room. This room was formerly used by Laboratory of Biology of Viruses. Also six new offices are being installed in former locker space, which was not being properly utilized. This new space will solve the existing problem of a need for offices for our professional investigators. Six used house trailers are on order to facilitate our field investigation expansion in and around Montgomery, Washington and Frederick counties of Maryland. These additional trailers will enable us to continue our program on cancer virus ecology.

FOREIGN SCIENTISTS

The Laboratory of Infectious Diseases has been fortunate in having the following foreign scientists working in our laboratory: Epidemiology Section - Dr. Fernand Bricout, sponsored by the National Centre of Training for Scientific Research, Paris, France. Medical Mycology Section - Dr. Nicolay Yelinov, Deputy Director, Lenigrad Chemical Pharmaceutical Institute, Leningrad, USSR. Dr. Maryjane K. Cook, Bacteriologist, Laboratory of Infectious Diseases, is stationed at Max Planck Institute, Tubingen, Germany, and is working on Influenza Viruses and studies of the development, sub-structure, and antigenicity of the para-influenza group.

TRAINING

Courses in outside training under sponsorship of the National Institutes of Health were completed by six professional and four laboratory personnel. These courses were approved on the basis of increasing our knowledge to better understand individual scientific projects.

CONCLUSION

The Laboratory of Infectious Diseases leadership in research on Infectious Diseases is recognized in all scientific circles. This reputation is due to the competent professional (some internationally known) and technical personnel who are devoted to their work. The Administrative Office of this laboratory is desirous of working with these scientists, realizing its role in providing for their needs. The facilities of the laboratory have expanded markedly this year, but we feel expansion in coming years will be greater in order to meet the demands of the public for infectious disease research.

PHS-NIH
Summary Statement
Calendar Year 1961

Laboratory of Infectious Diseases, NIAID

Administrative Office

Summarization of part of the honors awarded Laboratory of Infectious Diseases scientists during the past year:

Dr. Robert J. Huebner

Carl Puckett Memorial Lecture - "Viruses, Common Colds and Pneumonia"
Oklahoma City, Oklahoma - January 17, 1961

National Institutes of Health Lecture - "Viruses, Common Colds and Cancer"
Bethesda, Maryland - March 15, 1961

Lectures - Harvard University - "New Areas of Investigation in Infectious Diseases. 1) Respiratory Viruses; 2) Viruses and Cancer"
Boston, Massachusetts - April 13, 1961

Chairman, Virus Delegation to Russia - April 23 - May 21, 1961

Appointed consultant to Illinois Center for Zoonoses Research - July, 1961

Appointed Chairman of Panel of Consultants at Conference "Ecology and Epidemiology" - Illinois Center for Zoonoses Research - September 28-29, 1961

Dr. Arthur Saz

Chairman, President's Fellowship Committee, American Society for Microbiology

Lecturer (Professor) in Botany, Howard University, Washington, D. C.

Appointed Research Associate at the Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

Dr. Wallace Rowe

Invited speaker, AVMA Symposium on Diseases of Laboratory Animals, Detroit, August, 1961.

Invited speaker, ICLA Symposium on Diseases of Laboratory Animals, Prague, Czechoslovakia, September, 1961. (Paper delivered in absentia)

Invited speaker, Francis Delafield Hospital, September 30, 1961

PHS-NIH
Summary Statement
Calendar Year 1961

Laboratory of Infectious Diseases, NIAID

Administrative Office

Invited speaker, Symposium in Tumor Viruses, Massachusetts General Hospital, October 16, 1961

Mary Scott Newbold lectures, Philadelphia Academy of Medicine, March, 1961

Dr. Roy Repaske

Elected Secretary of the Division of the American Society for Microbiology

Invited to 5th International Biochemical Congress, Moscow (August, 1961)

Appointed a consultant for the Martin Company, Baltimore, in regard to an aspect of their space research program

Dr. Arthur Schade

Invited to participate in the 5th International Congress of Biochemistry, Moscow, August 10-16, 1961

Invited to participate in the 8th Congress of the European Society of Hematology, Vienna, August 28 - September 2, 1961

Dr. Benjamin Prescott

Invited to participate in "Symposium uber experimentelle und klinische Pharmakologie der Antibiotica", in Aachen, Germany, May 18-19, 1961

Participated in 2nd Congresso Latinamericano y l Nacional de Microbiologica in San Jose, Costa Rica, December 10-17, 1961

Dr. Chester W. Emmons

Appointed to W.H.O. Expert Advisory Panel on Parasitic Diseases for a term of five years

Appointed on Scientific Advisory Committee, Institute of Microbiology, Rutgers The State University, New Brunswick, New Jersey, for a term of three years

PHS-NIH
Summary Statement
Calendar Year 1961

Laboratory of Infectious Diseases, NIAID

Administrative Office

Member of Committee on National Index of Fungus Cultures, Quartermaster Research and Development, National Academy - Research Council, Natick, Massachusetts, for a term expiring June, 1963

Special Lecturer of Medical Mycology, G. W. University, Fiscal Year beginning August, 1960

Elected Councilor-at-large, American Society of Microbiologists, July, 1960

Appointed Board Member of the American Academy of Microbiology for a three year term

Chairman, Standards and Examinations Committee for Mycology, American Academy of Microbiology

Member of Study section of Mycology and Bacterial Diseases, NIH

Annual Lecture of the Mycological Society of America at Purdue University, August, 1961

Invitational Lecture, Japanese Mycological Society at Sendai, Japan

Presentation of two papers entitled: "Pulmonary Crytococcosis" and "The Fungi of chromoblastomycosis" at the 11 Congreso Latinamericano y 1 Nacional de Microbiologia, San Jose, Costa Rica, December, 1961

Served on Editorial Boards of: Mycologia, Journal of Bacteriology, Journal of Antibiotics and Chemotherapy, Mycopathologia

Dr. Karl Johnson

Presentation of paper entitled "Enteroviruses" at the Annual Meeting of the American Public Health Association, November 13, 1961, Detroit, Michigan

Invited to lecture New York Academy of Medicine, February, 1962

Pariticpant: Boehaave Post-Graduate Course on Respiratory Viral Infections Leiden, Holland, April, 1962

Invited to lecture Dartmouth Medical School, Hanover, New Hampshire, November 30, 1961

PHS-NIH
Summary Statement
Calendar Year 1961

Laboratory of Infectious Diseases, NIAID

Administrative Office

Presentation of paper entitled "Epidemic of Coe viruses (Coxsackie A-21) in Military Recruits" at the Annual Meeting of the American Epidemiological Society, April, 1961, Atlanta, Georgia

Co-author on "A Manual of New Viruses Causing Respiratory Illnesses in Man" Prepared for the Commission of Acute Respiratory Diseases of Armed Forces Epidemiological Board, 1961

Dr. Robert M. Chanock

Member of U.S. delegation on Virology which visited the USSR, April and May, 1961

Invited speaker at 1st International Congress on Respiratory Tract Diseases of Virus and Rickettsial Origin, Prague, May, 1961

Member of the virus study group of W.H.O. to advise on viral reference reagents, virus nomenclature, and the role of W.H.O. in virus research, August, 1961

Invited to lecture at 3rd Symposium of Perspectives in Virology, February, 1962

Participant in Symposium on Viral Respiratory Diseases, American Public Health Association Annual Meeting at Detroit, November, 1961

Invited to lecture at Boerhaave Postgraduate Course on Respiratory Viral Diseases, Leiden, Holland, April, 1962

Invited to participate in Symposium on Viral Respiratory Diseases, New York Academy of Medicine, February, 1962

Presented a paper on "Ecology of eaton agent and cold agglutinen positive a typical pneumonia" at the American Epidemiological Society, April, 1961

Co-author on "A manual of new viruses causing respiratory illness in man", prepared for the Commission on Acute Respiratory Diseases of the Armed Forces Epidemiology

Dr. H. F. Hasenclever

Presentation of a lecture on "Studies on Immunity to Coccidioidomycosis" at the Eleventh Annual Southwestern Conference on Diseases in Nature Transmissible to Man

PHS-NIH
Summary Statement
Calendar Year 1961

Laboratory of Infectious Diseases, NIAID

Administrative Assistant

Presentation of a lecture entitled: "Pathogenicity of Candida albicans. Antigenic Groups A and B." Round table mechanisms of Fungal Pathogenicity, 1961, at the annual meeting of the American Society for Microbiology

Presentation of a lecture entitled: "The prevalence of Histoplasmosis in Eastern United States", at the Potomac Chapter, American College of Chest Physicians, annual meeting, October, 1961

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

On the credit side we have been able to develop satisfactorily *in vitro* survey tests for mouse hepatitis, PVM, Theiler's virus, and LCM, thus adding these to our test battery which already included tests for polyoma, K virus, mouse adenovirus and mouse salivary gland virus. Thus by working with commercial producers of laboratory mice we hope to achieve rather soon supplies of mice that are free of infections with these agents.

I. Studies of Relationships of Specific Mouse Viruses

Immunological studies of mouse reoviruses showed them to be indistinguishable from those isolated previously from man, cattle and monkeys. An interesting sidelight was the demonstration that Stanley's encephalo-hepatitis virus is identical with reovirus type 3 and that the various other hepato-encephalitis agents isolated from mice and described by Cheever and others are serologically related to MHV and other strains of hepatitis virus.

Of special interest was the natural infection exemplified by an indigenous mouse hepatitis virus which invariably killed uninfected newborns from mice obtained from an SPF colony.

II. Use of SPF and Germ-Free Mice in Cancer Research

Th[illegible] to [illegible] great [illegible] progress in the use of susceptible virus-free SPF mice and germ-free mice. The main difficulty with the latter is that they are expensive and not available in large numbers. The SPF mice, as suggested above, are of value only when they can be kept in isolated virus-proof quarters, which currently are not available in our virus building.

We have tested two methods of isolation-both with considerable success. The first method made use of isolated house trailers which are chemically sterilized between experiments. These have been very useful for studies of polyoma virus in such field specimens as mouse tissues, excreta and mouse contaminated grain.

Recently, successful experiments have been performed in the Lobund plastic isolators. Although these require much attention and high caliber animal attendants, they may well be the answer to the production of pedigreed, clean and certified cancer virus pools - a prime requirement in cancer virology just as it is for respiratory virology.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

Virus and Epidemiology Sections

1.0 Goals

The work of these two sections continued to be aimed at better definition and understanding of numerous human and animal viral infections, particularly of their roles as etiologic agents in respiratory disease, cancer and birth defects. In other words, our chief concern is to find the true natural history of viruses, and define their importance in various disease states. In order to do this it has been necessary to develop appropriate tests and survey methods and in 1961 we were able to achieve comprehensive surveys of viral infections not only in man but also special studies of domestic and commensal animals. We have continued field and clinical studies in collaboration with research personnel of the Bureau of Medicine and Surgery, U. S. Navy; D. C. Children's Hospital Research Foundation; the D. C. Welfare Department; the National Institute of Neurological Diseases and Blindness; the National Cancer Institute; and the Laboratory of Clinical Investigations of the National Institute of Allergy and Infectious Diseases.

For the third straight year these studies have yielded over 50 scientific publications. Thirty papers reported studies on etiology, therapy and pathogenesis of respiratory agents and 14 were concerned with various aspects of cancer viruses.

An inclusive detailed review of each of our publications would take too much space. I will therefore briefly highlight the more important discoveries, and indicate future trends.

2.0 Respiratory Virus Disease Studies

2.1 NIAID as a Center for A National Program for Prevention of Acute Respiratory Diseases.

The enormous complexity of the acute respiratory disease problem requires that a concerted national program be set up for its final solution. Almost none of the numerous causative agents respond to antibiotics; thus efforts to prepare vaccines designed to modify or prevent the effects of 50 to 100 different viruses involved represents a task many times greater in magnitude than that which is required for the control of poliomyelitis. It is a problem that for logical and logistic reasons must be centered in a national laboratory such as NIAID, and the NIH.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

2.2 Delineation of Newly Recognized Causes of Acute Respiratory Disease

. Previous annual reports and publications have documented the key role of LID scientists in the discovery of adenoviruses, para influenza viruses and respiratory syncytial viruses, and in defining their importance in causing respiratory illnesses, especially in children. In 1961 the role of these agents in causing [illegible] per cent of the acute respiratory illness of infants and children was confirmed. In addition, the contribution of the other viruses (Coxsackie and ECHO viruses) to the acute respiratory syndrome was partly elucidated. Studies of outbreaks of Coe virus (Coxsackie A-21) in military recruits, Pett (Coxsackie A-24), Coxsackie B 3 and 5 viruses suggest that these enteroviruses (and the unclassified ones similar to the British Salisbury viruses) will soon be found to contribute significantly to the overall respiratory disease problem.

2.3 Vaccine Prevention of Acute Respiratory Diseases

Acute respiratory disease undoubtedly still represents the largest single source of human morbidity. The causative roles of the agents mentioned above together with influenza viruses and the PPLO (Eaton) agent is now sufficiently clear to provide a substantial reason for attempting to apply this information in efforts to control the diseases caused by them.

Known agents probably account for more than 70 per cent of the undifferentiated acute respiratory illnesses in children and as much as 40 per cent in adults. Prospects seem good for eventually preventing these infections with the use of either living attenuated or inactivated vaccines similar to the poliomyelitis and measles vaccines now in use. The many different viruses and other agents involved in respiratory illnesses renders the development and testing of experimental vaccines an expensive task, but the attempt must be made.

In 1961, therefore, Dr. Bell and associates in LID continued to test several experimental respiratory vaccines in Junior Village* children. The vaccines used incorporated inactivated antigens from adenoviruses and para-influenza but they were found to be too low in potency - producing neither satisfactory antibody responses or protection during outbreaks of these viruses.

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* Junior Village is a D. C. Welfare Department Institution for temporarily displaced children. We have studied viral infections and illnesses in this population for five years. It has been a fruitful study - yielding valuable data on viral illnesses much of which has already been reported; however much remains to be analyzed.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

A hopeful note was, however, achieved. Although an inactivated measles vaccine failed, a subsequent test of live attenuated vaccine was almost 100 per cent effective in preventing measles during an outbreak at Junior Village. This result together with other successes in preventing influenza and poliomyelitis in Junior Village with commercially produced vaccines, offers hope that useful viral vaccines can be prepared and that they can be successfully tested in Junior Village.

2.4 Antibiotics in Therapy and Prevention of Acute Respiratory Diseases

We completed studies on the value of tetracyclines in the treatment of primary atypical pneumonia due to Eaton's (PPLO) agent in adults and on the value of oral penicillin in preventing acute febrile respiratory illnesses in children. The first study of pneumonia which was carried out in Marine recruits at Parris Island (in collaboration with the Bureau of Medicine and Surgery, U.S.N.) showed that declomycin, a synthetic tetracycline, was very effective in the treatment of primary atypical pneumonia, reducing significantly the duration of fever, pneumonitis and hospitalization.

The daily use of oral penicillin for nearly a year in a proportion of children at Junior Village failed to significantly alter the overall illness rate despite the fact that hemolytic streptococcal infections were virtually eliminated from the throats of those in the test group. Only during brief outbreaks of several typable hemolytic streptococci was it possible to show any measurable beneficial effects from the drug. No effect was observed on viral infections.

Before the end of the fiscal year data on the prophylactic value of tetracyclines on the incidence and severity of respiratory illnesses should be available. Prospects for a measurable effect seems good because of the considerable amount of respiratory illness believed to be due to the Eaton (PPLO) agent.

2.5 Studies of Respiratory Viruses in Human Volunteers

Many studies of numerous respiratory agents (viruses and PPLO) in volunteers are scheduled to be carried out in cooperation with the Laboratory of Clinical Investigations and the Federal Bureau of Prisons. To date studies of the pathogenesis of several para-influenzas, RS, and Coe (Coxsackie A-21) viruses, and Eaton's PAP agent grown in tissue cultures have been completed. All were found to cause acute respiratory illnesses. Eaton's agent produced not only primary atypical pneumonia but also middle ear infections; in the latter case providing one of the first specific clues to the cause of nonbacterial serous myringitis.

One of the more interesting findings in the volunteer studies was the fact that many of the viruses tested has produced illnesses despite the presence of pre-existing antibodies; but the antibodies also were responsible for the general mildness of the illnesses observed.

Studies in volunteers of Coxsackie A-21 virus (a Coe-like strain isolated from a military recruit) revealed that growth of this enterovirus was almost entirely restricted to upper respiratory tract. This is interesting since other Coxsackie A-21 strains are usually found in the stools of children. Carefully designed volunteer studies provide a wealth of significant information on host-agent relationships, information unattainable in any other way. Hence they will be continued and expanded.

Importance in Vaccine Development. One of the chief purposes of the volunteer study program is to test live viruses for attenuation and for inclusion as candidates in experimental vaccines. Of course, killed vaccines can also be tested in volunteers - their efficacy in the volunteers can be measured by challenge with an antigenic variant of the [illegible] by LID scientists in developing the first effective adenovirus vaccine.

2. PPLO (Eaton Agent), RS and Para-influenza Viruses as Causes of Severe Respiratory Illness and Pneumonia.

The most significant finding since the beginning of our studies on Eaton's "virus" (Primary Atypical Pneumonia) was the demonstration that it is in fact not a virus but a PPLO (pleuropneumonia-like organism) which can be grown in artificial media. Although Marmion in England first developed evidence that this might be the case, the first cultivation of the organism in artificial media and the demonstration of its identity with the Eaton agent was first achieved in this laboratory by Dr. Chanock and his colleagues with an assist from Dr. Hayflick of the Wistar Institute.

This discovery is exciting for three reasons. First it opens the door to the development of simple methods for diagnosing PPLO. Secondly, it provides a ready means for production of potent vaccine. Thirdly, it suggests that besides the Eaton agent, other different PPLO's can be expected to turn up as causes of acute respiratory illnesses.

The fact that pneumonia due to the Eaton agent responds readily to tetracyclin therapy (see reports from this laboratory and the USN Bureau of Medicines and Surgery) and the fact that PPLO's are known to be generally very susceptible to broad spectrum antibiotics furnished previously undreamed of possibilities in the therapy of respiratory illnesses. The main job to be done now is to determine the relative contribution of PPLO's to the total acute respiratory disease syndrome in children and in adults. This job requires that our respiratory unit must develop new skills in and acquire new equipment and personnel trained to work with PPLO's.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

The importance of RS and para-influenza virus infections in childhood pneumonias and other lower respiratory illnesses was defined further in 1961. Large outbreaks of both continued to occur in our study populations and detailed studies have now been fully reported. Reports from other laboratories serve to confirm our finding that up to [illegible] per cent of the severe febrile respiratory illnesses of children are caused by Eaton's PPLO agent, RS virus, adenoviruses, influenza and para-influenza viruses, all considered together.

[illegible] Enteroviruses as additional causes of acute respiratory disease.
In children.

The enteroviruses (Polioviruses, Coxsackie and ECHO viruses) are well known as causes of certain specific illnesses such as poliomyelitis, herpangina, pleurodynia, aseptic meningitis and myocarditis. Less well appreciated are their importance in causing acute upper respiratory illnesses. It is evident from our long term studies of Junior Village children (where we observed nearly 2,000 persons infected with many different enteroviruses) that acute respiratory illnesses often with fever represent the most common clinical manifestations of most enteroviruses. Recently using more sensitive tissue culture isolation procedures, we have encountered numerous additional viruses having the properties of Coxsackie A viruses. The most common group of similar isolates are related to Coxsackie A-24 Pett virus (isolated several years ago by virologists in LCI) which is related to Coxsackie A-24 virus.

In adults.

Information on the role of enteroviruses as an etiologic agent of adult respiratory illness, particularly the common cold syndrome has been derived chiefly from outbreaks of ECHO 28 (2060 virus) reported at different times chiefly from [illegible] by [illegible] several years ago and in [illegible] by Hamre and the outbreaks of Coxsackie A-21 in Marines observed last year by Johnson, Mufson and Chanock from LID.

Recently, Johnson and Chanock used special tissue culture techniques developed by Tyrrell to demonstrate "Salisbury" type viruses. It appears that these can be recovered from about [illegible] to [illegible] per cent of adults with mild respiratory illnesses, thus providing new avenues for study of the "common cold". Studies of the effect of the "Salisbury Rhinoviruses" in human volunteers by Tyrrell and Andrewes in England suggest that they are indeed responsible for common colds. Recently Hamre recovered 24 similar strains from [illegible] cases of mild respiratory illnesses. However, many carefully designed epidemiologic studies will be needed to establish their actual importance in different groups and different ages. In LID we are planning extensive sero-epidemiologic and longitudinal virus isolation studies to evaluate the importance in respiratory disease not only of the newer enteroviruses but also of the well established enteroviruses which have similar properties and potentialities.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

1. New Adenoviruses and Enteroviruses

During the past year LID scientists discovered and reported a number of new representatives of the enteroviruses and adenoviruses. In nearly every [illegible] recovered from children in Junior Village, and data show that [illegible]; however, their importance as causes of illness remains to be determined.

These viruses were segregated from the known agents by Rosen using an interesting new approach. A pioneer in the development of viral hemagglutination tests, Rosen collected hundreds of isolates of adenoviruses and enteroviruses and reoviruses. Using specific hemagglutination and hemagglutination inhibition techniques (most of them newly developed by Rosen himself), he was [illegible] to identify those that fell into previously classified categories and segregate those that didn't into their separate new categories. These major technical breakthroughs represent one of the reasons for LID's pre-eminence in viral diagnosis and classification, and currently [illegible] hemagglutination and hemagglutination inhibition techniques developed by Rosen are being used by many laboratory workers, including his colleagues in LID - namely, Chanock, Johnson, Rowe, [illegible], and Sever.

2. LID as a Viral Diagnostic and Reference Center for Respiratory and Other Viruses

Standard Diagnostic and Typing Serums - During 1961, LID scientists were appointed project directors on three large commercial contracts for production of standard viral reagents; Dr. Huebner was asked to supervise the production of [illegible] adenoviruses 1 to 18 by the NIAID Adenovirus Committee and diagnostic antigens by the Cerebral Palsy Study Group of NINDB. Similarly, Dr. Rowe was asked by the Cancer-Virus Panel of NCI to serve as project director for the production of [illegible]. More recently, Drs. Chanock, [illegible] respiratory virus panel of NIAID and the [illegible] Committee of NCI to prepare reports and specifications for additional viral reagents. The laboratory has accepted these responsibilities not because these reagents are essential to furthering LID research but largely because of a sense of duty, stemming from the realization that optimum national progress in research on virus diseases will be delayed until these necessary resources are available to virologists everywhere. However, these responsibilities involve not only much work on the part of our personnel, but also utilize a significant part of the LID budget.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

The ability to efficiently handle more than [illegible] viral agents of man and animals requires a staff that is not only exceptionally capable, but also conscientious and responsible, motivated by a primary concern for [illegible] programs in their research as compared to their own private research interests.

Such a staff is not only exceptional, but is increasingly rare.

3.1 Diagnostic Antigens for New Viruses

During 1961 additional [illegible] viruses and enteroviruses were characterized by LID scientists and accepted for classification by the respective national committees. Many additional complement-fixing and hemagglutination antigens have therefore been prepared according to our specifications on the NIAID contract (a total of [illegible] CF and [illegible] HI antigens are now available).

3.2 New Micro-Serologic Test - a significant technical advance

In 1961 virtually all of the LID serology, CF, HI and metabolic inhibition (neutralization) tests were routinely performed by means of a micro-technique adapted by Dr. John Sever from a Hungarian (Takatsy) procedure. The quantities of antigen, serum and other reagents used in this test are one-eighth to one-tenth the amounts used in conventional tests. Careful evaluation of numerous antigens suggests that agreement with conventional procedures is approximately 90 per cent. Reproducibility is also equivalent. This new technique represents a real breakthrough, since it permits 8 times more tests with costly antigens and precious serums than could [illegible]. Thus our antigens which to date cost $300,000 to produce on contract are now worth 2.4 million.

Equally important has been the application of this technique to the diagnosis of extraneous mouse viruses by our oncology study group. Thus with 0.1 ml of serum taken from the inner canthus of laboratory or wild mice we can perform diagnostic serology in CF and HI versus 10 to 20 mouse viruses.

3.3 Fluorescent Antibody Studies of Eaton's PPLO Agent Grown on Acellular Media

Another technical step forward was of course the growth of the Eaton primary atypical pneumonia (PAP) agent on acellular bacteriological media. But of equivalent importance was the development by Chanock of a test for antibody responses against PAP using colonies of Eaton's agent grown on solid agar as antigen in the Coon's fluorescent antibody procedure. Paired serums from patients with PAP taken in five separate years showed in every case significant antibody rises in this new system.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

[illegible] Sero-Epidemiology of Human Viral Diseases

During 1961 antigens against more than 100 common viruses (6 myxoviruses, [illegible] adenoviruses, RS, 3 reo viruses, 19 entero viruses, and 10 mouse viruses) were evaluated in the micro-CF and micro-HI test systems.

This program represents collaboration between the cerebral palsy study group of NINDB and all of the research units in the Virus and Epidemiology Sections of LID.

Extensive serologic-epidemiologic surveys are now in progress on the prevalence of antibody responses against more than 100 viruses in women giving birth to abnormal babies, and in children and adults with acute respiratory illnesses, neurological diseases, and cancer. The prevalence of specific adenovirus and myxovirus antibodies in sick children at Junior Village and Children's Hospital is now under study, thus adding a new dimension to our knowledge of the natural history and pathogenesis of these agents.

Similarly serological surveys of these populations have revealed new and wholly unexpected information on the enteroviruses. Previous data from Junior Village which suggested that enteroviruses were important causes of acute respiratory illness, and that this may be the most frequent illness caused by enteroviruses, was confirmed by serologic studies of patients from D. C. Children's Hospital. These tests showed in 1961 that enterovirus infections in children with acute respiratory disease were eight times as frequent as in age-matched control children without respiratory illness. Extensive serologic studies are now in progress to evaluate the relative contributions of enterovirus infections to human disease. Over [illegible] paired sera are available from respiratory disease and control patients collected during the past two to five years from Children's Hospital, Junior Village, and Camp Lejeune. Similarly, serial specimens are available on 75 children with leukemia plus 75 controls and perhaps 10,000 pregnant women who have not given birth. Since these and other serum specimens are obtained from all parts of the United States, during several years (and from other countries as well) we should obtain valuable information on the regional occurrence of numerous viruses in different age groups and at different times; information thus far available to a meager extent for only a few viruses - namely, poliovirus and influenza.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

Studies of Cancer Viruses

Studies of animal cancer viruses have proceeded satisfactorily both in the field and in the laboratory. Perhaps our most important function is to focus attention on the importance and feasibility of studying the occurrence and distribution of cancer viruses in nature, to [illegible] laboratory tools which make such studies possible, and to continue to help define the extraneous viruses in cancer virus study systems which are producing almost insuperable obstacles to the development of reliable information about cancer viruses.

Studies of the Natural History of Polyoma Virus

The natural cycle of polyoma virus was found to be well established in *Mus musculus* living in farm, industrial ecologies in Maryland and in Indiana. There is extensive evidence that the age-old association of the house mouse first mentioned in relation to grain storage in the Mediterranean area (recorded in ancient Egyptian and Greek records and the Old Testament) now persists in modern grain storage facilities, on farms and in central depots all over the world. Although *Mus musculus* is not indigenous to all parts of the world, it originated in southern Asia and the Middle East, from whence it was carried along lines of trade to other parts of the world reaching northern Europe and England by 900 A.D. and becoming well established in the New World only within the last two centuries. Studies in southern England and nationwide surveys in the United States show that infestation of grain storage areas [illegible], almost universal, and frequently very intensive. For instance, a recent 29 state U.S. survey revealed an average count of [illegible] mouse pellets per peck (about 15 lbs) of shelled corn.

LID studies in the field to date show that polyoma virus infection [illegible] in *Mus musculus* on [illegible] dairy farms, [illegible] Maryland [illegible] grain [illegible] Indiana grain mills. Polyoma virus [illegible] for livestock [illegible] (but also for [illegible] consumption) wherever such grains showed [illegible] of mouse contamination and where the [illegible] found [illegible] for polyoma. This was not surprising, since infected mice are known to excrete large amounts of virus for long periods in the urine and infestation with polyoma-positive mice was very heavy in most of the ecology studies. Thus, as reported last year, the polyoma cancer virus is widely distributed in all the ecologies in which commensal house mice live - in laboratories, in production colonies of laboratory mice, in densely populated urban tenements, and in rural environments.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

5.2 Importance of Cereal Grain Contamination by Mouse Viruses

It seems quite likely that widespread contamination of grain usually used uncooked for feeding laboratory mice and which is generally available to wild mice as well may be responsible for the widespread infection of mice with polyoma virus.

Of equal interest perhaps is the fact that other viruses can also be demonstrated in *Mus musculus* living in human and animal grain supplies. These include mouse hepatitis, reovirus 3, lymphocytic choriomeningitis, salivary gland virus, and K virus. Mouse leukemia and mammary cancer virus probably occurs in grain since previous studies have demonstrated both viruses in wild mice; however, methods for detection of these viruses are not yet adapted for survey purposes.

The significance of these findings in relation to human or domestic animal diseases can only be surmised at present. However, it is clear contacts between these species with house mice and their viruses is frequent, intimate and possibly occasionally extensive.

5.3 Laboratory Studies of Animal Cancer Viruses

LID studies of polyoma virus are largely centered in the field. In the laboratory most of our efforts are focused on attempts to adapt various mouse and avian leukemia viruses to *in vitro* study systems. New methods developed for avian leukemia virus by Beard, Rubin and Baluda suggest that tests suitable for epidemiologic surveys will soon be available for chicken lymphomatosis and myeloblastosis viruses and for avian sarcoma. Attempts to adapt rabbit and human papilloma viruses to tissue culture systems by White, a visiting scientist from Australia, failed; however, specific and useful complement fixation and neutralization tests for rabbit papilloma virus were obtained and evaluated during 1961.

5.4 Extraneous Viruses as Background Noise in Cancer-Virus Study Systems

This problem has if anything become worse. Additional viruses were found in production colonies of laboratory mice, including PVM, new strains of hepatitis and Riley's plasma agent. For instance, Riley's agent, together with reovirus 3 and hepatitis virus turned up successively in our passage lines of Moloney's leukemia.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

7.G Studies of Domestic Animal Viruses

Domestic animals (cattle, sheep , swine) have many opportunies to transmit infectious agents to man. Itis not our aim to resume studies of old zoonoses such as Q fever, psittacosis, or swine influenza.

However, during the last four years, Drs. Abinanti and Rosen have demonstrated a number of viruses closely related to human agents in the respiratory and enteric system of cattle (i.e. parainfluenza 3, and reoviruses types 1, 2 and 3) which deserve study. Recently other workers have isolated adenoviruses and numerous agents that have the properties of enteroviruses from cattle.

In 1961, Dr. Abinanti reported on a new hemadsorbing enterovirus from cattle, and with Dr. Rosen succeeded in also isolating numerous enteroviruses from cattle feces.

It now appears that the serotypes of bovine enteroviruses may be quite numerous (as are the human and simian agents). Before rational and systematic investigations of these agents will be possible, they must be characterized, a system of classification devised and then tested for immunologic relationships with the already classified human and simian viruses. This problem is so critical and its importance so obvious that Dr. Abinanti working with Rosen and Johnson in this laboratory plans to devote the next year to efforts designed to set up the first systematic classification of bovine enteroviruses.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

Medical Mycology Section

The Mycology Section is engaged in a multidisciplinary and multifaceted research program on Mycotic Diseases. In the field the work is focused on isolation and identification of new fungi and on ecologic studies on various fungi known to be pathogenic for man. The studies of new fungi are done in collaboration with the hospitals and diagnostic laboratories; the ecologic studies in collaboration with the Rabies Control Unit, Trinidad, West Indies. Additional field programs are planned. New antimycotic drugs and antibiotics are tested for efficiency and safety, in collaboration with the Medical Physiological Bacteriology section of LID and withthe Laboratory of Clinical Investigations. The clinical and field studies are supported and supplemented by basic work in laboratory on the physiology, toxicity and immunology of pathogenic fungi. The search continues for serological methods capable of recognizing mycotic infections in humans and in various animal hosts, and for improved techniques designed for growing and identifying fungi.

The Mycology Section reported 11 projects for calendar year 1961. Results were published in 10 papers and 5 additional papers are in the press. Three book reviews were published.

C. W. Emmons has isolated Histoplasma capsulatum in Washington, D. C. for the first time from a congested urban area, from soil adjacent to houses in Trinidad, W. I., which harbor bats, and from soil under roosting sites of starlings. He has continued studies of the new antibiotic X-5079C in order to determine its activity when used in combination with Amphotericin B. He has described with Dr. Charles Bridges of the Agricultural and Mechanical College, Texas, a new disease in horses caused by Entomophthora coronata and described the fungus Hyphomyces destruens from a disease of horses.

G. W. Lones has investigated the quantitative relationships of X5079C with respect to fungistatic action on Histoplasma capsulatum and Blastomyces dermatitidis in the two morphological modifications. The drug has been found not to inhibit respiration on ammonia assimilation in Histoplasma capsulatum. However, there was evidence that nucleic acid synthesis is inhibited by X5079C to a greater extent than protein synthesis. He has maintained two strains of Coccidioides immitis in the spherule phase and found that the mycelial phase of this organism grows rapidly on mannitol as a source of carbon and the spherule phase not at all. The basis for the nutritional difference in the two forms is being examined.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

Medical Mycology Section

Dr. H. F. Hasenclever has immunized mice against challenge with *Coccidioides immitis* and *Candida albicans* by injection of nonviable *C. immitis* or *Candida albicans* cells. Mice infected with a sublethal dose of injected with nonviable cells of *Coccidioides immitis*, *Salmonella lipopolysaccharides*, and other species of *Candida* are more resistant to intravenous challenge with *C. albicans* than normal mice.

With V. T. Andriole he demonstrated increased susceptibility to species of *Candida* in mice exposed to X-radiation or made diabetic with alloxan. He has demonstrated two antigenic groups within the species *Candida albicans*, one being similar antigenically to *C. tropicalis* and the other to *C. stellatoidea*.

Dr. John Bennett, working with Dr. Hasenclever, has produced a highly potent anticryptococcal rabbit serum with which cryptococcal polysaccharides can be demonstrated in the body fluids of patients.

Dr. Donald Summers, working with Dr. Hasenclever, is investigating the effects of alteration of macrophages upon subsequent ingestion of fungus cells.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

Medical and Physiological Bacteriology Section
Dr. A. K. Saz

Introduction

The Medical and Physiological Bacteriology Section had an active and satisfying year. Definitive progress has been made on all problems under investigation by the staff of the section and the cross-fertilization occurring as a result of many individuals working on different problems, though all concerned with basic cell biology, has proved of inestimable value to us all. The professional relationships among the various members of the section have been of a high order and have contributed to a most satisfactory professional atmosphere. The doctoral level personnel of the section however, are disappointed (as we have been for several years now) that there is still no microbial genetics program in the section. Each project leader could benefit by informal discussions with a geneticist. Beyond this, however, is the awareness that the section is not truly contemporary without this most important discipline represented in our program. Representation will be made separately, once again as for the last four years, for funds and space for a microbial genetics program within this section.

A highly interesting observation concerning staphylococcal penicillinase has been made. It seems probable that this enzyme is a non-specific cyclic peptidase, splitting various cyclic peptides to straight chain compounds -- this observation offers at least a partial answer to the hitherto disturbing question of the role and function of penicillinase in bacteria in their natural habitat. Under normal ecological conditions, it is highly unlikely that bacteria ever come into contact with penicillin, hence the value of the possession of penicillinase to the total economy of the cell has been dubious. By offering the possibility that penicillinase is primarily a specialized peptidase, its role is conceivably enlarged to a more important function in cellular metabolism.

Significant observations have been made on the problem dealing with the antibacterial activity of marine waters. It is now quite clear that the activity is due to a large organic molecule with molecular weight greater than 10,000 rather than due to the inorganic ion content of the sea water. This observation, obtained as a result of exhaustive dialysis experiments, settles in large part a controversy which has been raging for 75 years amongst biological oceanographers. Partial isolation and purification of the active factor has been obtained. Further, the activity seems to be quite specific for gram-positive bacteria, thus offering an attractive hypothesis in explanation of the fact that the vast majority of bacterial genera in the oceans are gram negative. The activity is always present in ocean water, varying only quantitatively and this finding answers in part the hitherto disturbing

observation that activity of raw sea water varies temporally and seasonally. From the point of view [illegible] antistaphylococcal factor, particularly [illegible] penicillinase producing staphylococci, this is, of course, decidedly advantageous.

Highly significant observations have been discovered this year dealing with metabolic differences occurring between iron-deficient and iron-sufficient strains of Staphylococcus aureus. The degree of iron depletion or enrichment was controlled by the ratio of free Fe to iron-bound siderophilin in the medium of growth of the organisms. Using radioactive tracers, it is now quite clear that iron-deficient cells lack the ability to metabolize the six carbon of glucose when compared with iron-sufficient cells and further that the deficient cells also have decreased ability to incorporate carbon into protoplasm. These observations are a highly important beginning in a project designed to understand the biology of staphylococci. Since production of the various staphyltoxins presumably mirrors the overall metabolic processes of the cells and since, as indicated above, iron plays such a highly important role in metabolism, the hope remains that by juggling iron availability *in vivo* (and this can be done by proper use of siderophilin), one will be able to render invading staphylococci less toxic.

In the development of the above thesis, it has been essential to devise methods for purification of [illegible] active siderophilin and for assying such activity. This has finally been done by various ingenious biochemical techniques and the problems can now be attacked forthrightly.

Preliminary clinical data indicate the efficacy of strain specific therapy for various antibiotic-refractory urinary tract infections and work will be continued on this important and aggravating infectious condition.

Study of the M antigen of Group A, beta-hemolytic streptococci has yielded a surprising but highly important dividend of basic significance. A controversy which has been going on for decades over whether synthesis of the cell wall in dividing bacteria occurs by random intercalation or at specifically polarized growing loci has been all but settled, at least for the Group A streptococci. Using, alternately, periods of growth in culture media with and without [illegible] labeled [illegible] M protein, the issue has been definitely decided in favor of the latter hypothesis, i.e. polarization. The observation is of significance to all studying cytokinesis and morphogenesis in bacteria and fungi as well as to those interested in cell wall synthesis in general. Interest, [illegible] cell wall has been high in recent years since many important inhibitors, including penicillin, presumably act by virtue in inhibition cell-wall synthesis.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

Further improvement of the "long-chain" test for streptococcal type specific antibody has been made and its simplicity and accuracy appear likely to make it a most valuable tool for the answering of many clinical and epidemiological studies. Currently, it is being used in the study of pre and post- isolation sera from a type 12 streptococcal outbreak among institutionalized nursery-school-age children.

The mechanism of the long-chain reaction has been under investigation and the possibility exists that the phenomenon is a form of agglutination requiring bivalent antibody and may not be enzymatic as suggested by other workers.

Other studies dealing with Group A streptococci include effect of M-protein on phagocytosis of streptococci by mouse leukocytes and, by use again of fluorescein-labelled antibody, the fate of phagocyte-ingested cells, and the conversion of streptococci to protoplasts.

Studies on the detoxification of toxic, potentially useful antimicrobials have continued. New chemical methods for treatment of isoniazid, streptomycin, kanamycin, cycloserine and Miracil D to render them less toxic have been devised with excellent results.

A number of new aromatic iodine compounds with low toxicity has been synthesized with marked in vivo parasiticidal activity. Newly synthesized, long-chain thiosemicarbazones and salts of thioxanthanones have passed initial screening by the Cancer Chemotherapy Screening Center as antitumor agents and further tests are inprogress. The same compounds are being tested for antileprotic and antiviral activity in man.

Of vast potential import, is the isolation, characterization and partial purification of antibacterial and antiviral substances from mollusks, particularly oysters. Two fractions, decidedly different in behavior on columns, but both presumably glycoproteins, have been found to be active against bacteria, including staphylococci and viruses (polyoma and influenza). The significance of this is obvious for an effective chemotherapy of small virus infections is of great moment.

Marked advances have been made in an understanding of the electron transport of Hydrogenomonas and as a consequence, relying on the thesis of comparative biochemistry, the main energy-yielding systems of mammalina tissues. The system in the autotrophic Hydrogenomonas is ideal for these studies since it is, in distinction to systems in all other forms studied, at least partially soluble. This fact obviates the extreme difficulty of working with particulate and hence highly complex material. As further indication of the relative simplicity of the complex in this organism has been the finding that it contains only one cytochrome. It now seems obvious that

flavoproteins are active in this system in the transport of elutions and a judicious thesis can be presented for the formation of reduced pyridine nucleotides via the hydrogenase system. However, a new intermediate, different from others heretofore described, apparently is involved in the formation of reduced pyridine nucleotide. This, of course, has vast theoretical ramifications and would indicate important differences between bacterial and human energy-yielding systems and might well indeed be a basis for selective attacks on bacteria by antibacterials.

Work on this system in the past has been hampered by the instability on extreme oxygen sensitivity of the enzymes involved. These limitations have now been overcome as a result of work performed during the past year and rapid progress is now possible.

Cell walls are the largest anatomical structure of bacterial cells. It is, therefore, surprising that until recently very little has been done in investigations which might lead to a better understanding of this structure. However, as a result of the observations indicating that various inhibitory substances including penicillin and cycloserine may be active inhibitors by virtue of interference with cell wall matabolism, new emphasis has been placed on this structure. Very recently, it has been reported that polymers of ribitol or glycerol phosphate, the so-called teichoic acid, represent 30-60% of the dry weight of the cell. Investigation into the possible relationship between penicillin-resistance and cell-wall metabolism and synthesis is obviously of prime importance. A new project in this section deals with the teichoic acids of penicillin-sensitive and -resistant staphylococci. This is an enormously complicated field and isolation, purification and assay of the acids present extremely difficult problems, Nevertheless, real progress has been made in elucidating the conditions necessary for extraction of these cell-wall components in active form and a study is under way looking toward the nature of the linkage of teichoic acid to the remainder of the cell wall.

An extremely important observation has been made indicating that staphylococcal teichoic acid is heterogeneous and the physio-chemical properties of these acids are being studied. This heterogeneity may be related to the immunological specificity of the staphylococci.

In the past year, Dr. Austin Shug, Mr. Coleman Seward, Mr. Esly Caldwell and Mr. George Canty have resigned from this section and Dr. Eskin Huff, Miss Harriet Milner, Mrs. Nancy Harriff Myers and Miss Collette Lizottee have joined the section.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

A. Studies on cellular and cell-free staphylococcal penicillinase.

The study of staphylococcal penicillinase has taken a new and interesting turn. The role of penicillinase in the natural ecology of those cells possessing this activity has been a matter of considerable interest to us for some time now. It seems quite clear that, au naturel, organisms simply do not come into contact with penicillin. No one has as yet shown that penicillin, or for that matter, any other antibiotic, is produced in the soil or other milieu, e.g. nasopharynx, throat, etc. As a consequence of this, the possession of penicillinase activity by any given strain, would seem to be of no profound survival value to the cell, were penicillin the sole substrate for the enzyme. Since penicillin is a cyclic dipeptide of D-valine and D-cysteine, the thought occurred to us that penicillinase might be a non-specific enzyme acting upon various cyclic peptides. We were fortunate at this time in being able to secure two cyrstalline cyclic peptides from Dr. M. Winitz, NCI, NIH who was interested in having these compounds tested for antibacterial activity. The compounds affected various organisms as if the peptides were penicillin. Penicillinase-producing strains were resistant to the compounds, sensitive strains were sensitive. Tests for hydrolysis of the peptides by the penicillinase preparation indicated that, indeed, hydrolysis occurred. Currently, by use of the fluorodinitrobenzene technique, we are attempting to pinpoint the locus(i) of cleavage.

This is an extremely interesting observation from a theoretical standpoint and it also offers some distinct practical possibilities. Since virtually all cyclic peptides isolated to date from biological sources possess viological activity (e.g. various antibiotics, vasopressin, oxytocin, etc.), widespread occurrence and availability of an enzyme of this type could have important clinical possibilities. In addition, screening of cyclic peptides for potential chemotherapeutic purposes against penicillinase-producing staphylococci will be made easier, should this hydrolysis prove to be general, since assay for hydrolysis takes approximately 5 minutes whereas growth-inhibitory assays take 24 hours. The search for penicillinase inhibitors has continued as detailed in the annual report for 1960. Thus far no compounds with activity greater than those previously reported have been found.

B. Antibacterial activity of sea water

Significant progress has been made on the investigation dealing with the antibacterial activity of sea water. The controversy which has been going on for 75 years over whether the observed lethal activity of sea waters for terrestrial bacteria is due to the inorganic ion content of the waters or is due to an organic constituent has been settled, at least in part. Exhaustive dialysis of sea water versus running tap or distilled water leaves a preparation remaining inside the dialysis bag with undiminished

activity. This would indicate that the putative substance has a molecular weight of greater than 10,000. Since it is highly improbable that an inorganic polymer of this size could occur under the conditions of the experiment, it seems safe to say that the compound is organic. The active material has been concentrated by flash evaporation and vacuum distillation and can be re-diluted to original activity. Concentrated material has been placed in an electrophoresis apparatus and some degree of resolution has been obtained.

The compound possesses strong specificity for gram-positive bacteria, since all six gram positive species tested were killed by the sea water or fractions of it while five genera of gram negatives were either uninhibited or even grew out in the waters. This observation offers an attractive explanation for the fact that the bulk of the bacterial genera in the oceans (greater than 95%) are gram negative.

From the biological point of view, another highly significant fact has been noted. It has been a disturbing observation that the antibacterial activity of sea water has waxed and waned in time. We have been able to show definitively that this difference is strictly quantitative rather than qualitative since all samples of sea water collected,are active when properly concentrated regardless of initial lack of activity of raw, untreated water. Ths observation lends distinct encouragement to the possibility that a compound can be isolated from sea water which has use in the treatment of bacterial infections and particularly those caused by penicillin-resistant staphylococci. It should be noted that, if anything, penicillin-resistant penicillinase-producing staphylococci are more sensitive to the lethal activity of sea water than are penicillin-sensitive staphylococci.

It will be of interest to determine whether sea water preparations, particularly when more progress is made on isolating and purifying the antibacterial substance, have any effect on viral multiplication.

C. Non-specific immunity associated with serum siderophilin and the iron metabolism of both pathogen and host.

Research on this project is oriented towards elucidation of the following problems: The contribution of blood serum siderophilin to the natural resistance of the host; the iron requirements of selected pathogens and the effect of the iron-chelating siderophilin on iron accumulation, growth, and metabolism of these pathogens; the factors leading to changes in concentration of both serum iron and siderophilin in circulating blood as a result of infection and other abnormal conditions; and the consequence of such changes on resistance to infection, iron absorption by the host, and anemia.

While these problems are all considered worthy of investigation, the severe inadequacy of staff available requires restriction of effort to limited, possible goals. Extending our investigations on Staph. aureus as adumbrated in our 1961 summary report (q.v.), we have established, among others, the following additional facts:

1) In growth studies with Staph. aureus we have observed that cells grown in high iron media accumulated increasing amounts of the metal while those grown in low iron media retained a constant low amount of iron throughout the growth period.

Although both types of cells reached their stationary phase of growth in approximately the same time of incubation, the cell population was usually ten-fold or more greater in the media with high iron than in the same media with low iron. Manometric experiments employing these high and low iron cells established that the previously reported metabolic differences of such cells are independent of the physiological age of the cells. Preliminary enzymatic analysis of cell-free extracts, particularly their DPNH oxidase and dehydrogenase activities, have been performed but precise quantitative evaluation awaits further study.

2) Following up on observations that iron-low cells underwent spontaneous lysis more rapidly than iron-rich cells, we analyzed extracts of both types of cells for possible nucleotide cell-wall precursors. No differences were detected between the extracts from the two types of cells.

3) Employing C^{14}-tagged glucose we have studied the metabolism of this substrate by high and low iron cells. Our results attest to an oxidative deficiency of iron-deficient cells and a lack of ability to oxidize or decarboxylate the moiety of the metabolized glucose containing the 6-C^{14} carbon. Not only is the oxidative capacity of iron-deficient cells impaired but also their ability to incorporate carbon into cell material when compared to iron-rich cells.

As opportunity permits, we plan to continue our investigation of the growth, metabolism, and enzymatic capabilities of S. aureus when grown under conditions approximating the in vivo state with especial regard to the effect of different serum-iron saturations on such characteristics. Emphasis will also be placed upon the biochemical mechanisms by which iron controls the overall metabolism of the organism. We are particularly interested in investigating both qualitatively and quantitatively the production of staphylotoxins by Staph. aureus grown in serum at high and low iron levels. This area of study has immediate clinical implications since the in vivo milieu often does provide, or can be made to provide, growth conditions for the bacterial pathogen favorable to high iron availability or to iron restriction. Additional biological features such as effectiveness of phagocytosis and possible differences in response to antibiotics of high and low-iron containing cells should be studied.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

D. Biochemistry of the acquisition of iron by mammalian tissues as mediated by the iron-binding protein, siderophilin.

Siderophilin is the specific iron-binding protein component of the circulatory system fluids, serum and lymph, and has already been shown to possess three biological functions: iron transport in the body, non-specific immunity against iron-dependent pathogenic bacteria, and inhibition of the production of lipid peroxide in the circulating blood. All three functions are ascribable to the specific iron-chelating capability of the protein. To study and exploit the properties of siderophilin for clinical use in infectious and constitutional diseases it is necessary to possess isolated siderophilin in native form and in quantity. During this year, we have continued our search for a simple, reproducible method to test for the "undenatured" and physiologically fully active preparation of isolated siderophilin. We have succeeded in the development of a liver-mince-iron uptake method which, when used in conjunction with our reticulocyte iron uptake procedure (See 1960 Project Report NIAID #63-B), establishes the physiological suitability of the isolated siderophilin for laboratory investigation and for clinical use. A comprehensive discussion of the several siderophilin analytical methods developed by this laboratory provided the substance of a paper given in Moscow at the Internationl Biochemical Congress while a detailed presentation of the new liver-mince test method formed the basis of a paper presented to the European Hematology Congress at Vienna.

Utilizing both our reticulocyte and liver-mince iron-uptake tests for evaluation of the adequacy of several isolated siderophilins, we concluded that siderophilin isolated through the use of Rivanol as precipitant is physiologically "denatured". Our evidence suggests that satisfactory siderophilin can be obtained by differential salt precipitation and column chromatographic procedures.

Studies with reticulocytes, liver-mince, and cells of Staph. aureus as iron absorbing agents from an iron-siderophilin source have established that reticulocytes and bone marrow possess a specific mechanism for the accumulation of iron from this protein chelate referable to the specificity of the protein molecule itself. Liver mince and staphylococcal cells depend for their iron on the natural dissociation of the metal-protein complex itself and take up the ionic iron thus made available.

We propose to continue our investigation of the chemistry of the iron-siderophilin complex; to extend our knowledge of the manner in which various cells and tissues satisfy their iron requirements through the intervention of iron-siderophilin and to make such applications of our test methods and to improve them so as to assure the production and availability of adequate quantities of 'undenatured' siderophilin for use by clinicians in their investigations.

E. Coliform and Pseudomonas urinary tract infections refractory to antibiotic drug therapy.

The objectives of this project are: to reinvestigate the isolation and development of bacteriophages specific for antibiotic-resistant strains of Escherichia coli, Aerobacter aerogenes, Proteus vulgaris, and Pseudomonas aeruginosa; to produce phage lysates of high titer and virulence; to introduce such lysates into the urinary tract including the kidney pelvis; and to follow the course of the infection and its treatment with careful medical and urological supervision. To investigate the use of lysozyme alone and in combination with ethylenediaminetetraacetic acid solutions in the urinary tract for treatment of Pseudomonas aeruginosa infections and to assess the effect of bacteriophage lysates plus appropriate lysozyme mixtures on these and coliform infections.

Based upon a previous experience with the successful use of specific Proteus bacteriophage lysate in the treatment of a urinary tract infection, we instituted a course of treatment recently for a patient suffering from a long standing (more than 7 years) chronic E. coli urinary tract infection involving 2 bacterial strains. Following introduction into the pelvis of each kidney of 10 ml of high titer lysate specific for the 2 E. coli strains, the patient over the past month has voided only sterile urine and has improved generally in health. Since the incidence of refractory Pseudomonas and Coliform urinary tract infections represents a significant proportion of the difficultly manageable medical problems facing the urologist today, we propose to pursue such investigations as will realize the objectives of this project.

F. The Biology of Group A Streptococci

Advance in knowledge of the pathogenesis of streptococcal disease, and particularly of its non-suppurative sequelae, has not been rapid despite a voluminous literature, one reason for this state may be the scarcity of recent basic investigations of streptococci - particularly in the direction of new techniques which might be applied to clinical and epidemiological studies as well as to investigations of phenomena common among bacteria. Accordingly, this unit in the past year has emphasized the continuation of basic studies of group A streptococci.

A study of the improvement of tests for streptococcal type-specific antibody was initiated last year and has been continued. One such test, the "long-chain reaction" previously described was simplified and improved through study of "time-dose" relationships and application of standard statistical methods. The results are described in a publication (J. Bact., 83, Jan. 1962). We expect the method to have general acceptance as a marked improvement over older techniques, and hope that it will be widely used to answer clinical and epidemiologic questions which have been

awaiting a suitable simple test. We are at present applying it to a study of pre- and post-isolation sera during a type-12 outbreak among institutionalized nursery-age children.

Our previous discovery that group A streptococci grow well in fluorescein-labelled homologous antibody has led to new information on bacterial cell wall formation. By growing the streptococci in such labelled antibody, the cell walls become labelled. When growth is continued after precipitation or inhibition of the antibody, cell wall subsequently formed is unlabelled. A complementary technique of initial growth in unlabelled antibody, followed by antibody precipitation, growth, and then fluorescent antibody, has also been used. "Old" and "new" cell wall have thus been differentiated visually for the first time by a cell-wall marker in a living system. The results show clearly that new bacterial cell wall is not formed by diffuse intercalation with old -- as believed by some investigators -- but instead is formed along an equatorial region of activity in each dividing cell. We anticipate wide application of this technique to the study of bacterial and fungal cytokinesis and morphogenesis. The method is described in a forthcoming publication. We are now applying it to bacteria other than streptococci; and are using similar labelling to visualize the effects of various antibiotics on synthesis of streptococcal cell wall components.

The same technique has been applied to study of the mechanism of the long-chain reaction. Long chains form in type-specific antibody and break up after antibody is depleted as growth continues. By using fluorescent antibody, we have shown that the site of chain fragmentation under these circumstances is in areas of new cell wall formation (e.g., not antibody-coated). In either group-specific antibody or univalent fragments of type-specific antibody, long chains are not formed --which means that there is frequent separation between cocci. When such antibodies are fluorescein-lablled and used as above, it can be seen that separation between cocci occurs as often at antibody-coated sites as at uncoated sites (e.g., "old" vs. "new" wall). These results show that complete or bivalent antibody is required for the long chain reaction and that such bivalent antibody must be type-specific. If univalent antibody inhibits an homologous enzyme antigen as well as does its parent bivalent antibody (as has been shown to be true in other systems), then it appears that the long chain phenomenon is a form of agglutination requiring bivalent antibody and may not be enzymatic as suggested by other workers. Why group-specific antibody fails to agglutinate similarly, however, is at present unexplained. Investigations on the mechanism of long-chaining are continued.

Previous studies of the effect of the type-specific antigen and presumptive virulence factor, M-protein, on phagocytosis of streptococci by mouse leukocytes, have been extended. Enhancement of phagocytosis in the presence of added homologous M-protein has now been noted for 5 different serologic types of group A streptococci. M-protein prepared by acid hy-

drolysis gives such results, but M prepared by the picrate method described last year does not. Further investigations of this phenomenon, in a system of human leukocytes, are in progress.

With our attention directed to cell wall and surface antigens, we have initiated studies of streptococcal phages. The nature of surface antigens may become more apparent if, by labelling and blocking techniques, it can be shown which of them contain receptors for phages. In addition, it is of interest to see if phage sensitivity or lysogenicity may be related to virulence or toxin production of the host streptococcus. To date, we have emphasized only development of special methods for working with streptococcal phages.

Other studies in progress include those on the intracellular fate of phagocytosed streptococci, making use of fluorescein-labelling methods we have developed. Similar techniques will be used to follow conversion of streptococci to protoplasts. In general, we feel that group A streptococci are organisms which are well suited to basic studies of many bacteriological phenomena in general, and in particular of those systems of importance in understanding streptococcal disease and its sequelae.

G. Detoxification of potential tuberculostatic, fungistatic, parasiticidal and viricidal agents.

Our continued studies in mice on the detoxification of toxic, chemotherapeutically active drugs such as isoniazid (INH), streptomycin (SM), kanamycin and cycloserine for tuberculosis; neomycin for other infections; complex salts of Miracil D with monocarboxylic aromatic acids, piperazine derivatives, aromatic iodine compounds, antihistamines and tetracycline derivatives for parasitic infections and numerous hydrazones for rat leprosy and viral infections and certain drugs (Amethopterin) in use in cancer therapy show that with the addition of certain adjuvants to these toxic drugs, appreciable reduction of acute and chronic toxicity is obtained. With streptomycin-isoniazid mixtures, 50 mg of sodium glucuronate and 50 mg glycine per 20 gram mouse permitted 37 of 40 DBA mice (93%) to tolerate the administration of 15 mg SM plus 8 mg INH. Mouse tolerance was thus increased up to three times the lethal dose. In addition, aromatic sulfonic acids tested showed excellent detoxifying action with lethal mixtures of SM-INH when administered simultaneously to mice. A 14 mg dose of calcium pantothenate or 25 mg of nicotinic acid permitted 98% and 93% survival of DBA mice respectively when 25 mg of kanamycin was administered daily for 30 days. With 35% glycerol formal as solvent, a lethal 10 mg dose of drug dissolved in 35% glycerol formal 72 of 80 mice (90%) survived. An iodide complex of Miracil D permitted mice to survive a 20 mg dose (4 x lethal dose).

H. Isolation, purification and characterization of antibacterial & antiviral substances from mollusks.

Several series of new, non-toxic, potential chemotherapeutic agents against bacterial, fungal, parasitic and viral infections have been synthesized. Further studies on the antimicrobial activity of substances from various mollusks are in progress. Two active fractions were isolated; one is active against bacteria *in vitro* and *in vivo*, while the second is active against viruses (polyoma and influenza). Chemical studies of the active fractions show the material to be protein in nature, presumably a glycoprotein since glucose is present in the molecule.

I. Potential fungistatic, parasiticidal, leprocidal and viricidal agents.

A number of aromatic iodine compounds with low toxicity have shown *in vivo* activity in parasitic infections. Several of the long chain thiosemicarbazones and salts of thioxanthanones have passed initial tests as antitumor agents and are being further evaluated by the Cancer Chemotherapy Screening Center. A number of the same thiosemicarbazones and hydrazones synthesized are being tested for activity against leprotic and viral infections in man.

J. The electron transport system in an autotrophic hydrogenomonad.

Observations and data resulting from basic studies on the biochemical pathway by which *Hydrogenomonas eutropha* oxidizes hydrogen could have far reaching implications, because aerobic oxidation of nutrients (whether hydrogen, lactic acid, glucose or some other substance) is a fundamental biochemical process of all oxygen-requiring organisms including humans. Data obtained with a bacterium would contribute significantly to the understanding of body chemistry, and any deviations observed in the bacterial pathway would suggest potential sites for attack by antibiotics or chemotherapeutic agents to destroy selectively bacteria in infected hosts.

It is true that closely related organisms have unique biochemical reactions peculiar to that group of organisms, but the underlying pattern of metabolism in living organisms generally follows the same course. Throughout evolution the same major selective environmental factors have influenced most living organisms, perpetuating those organisms whose genetic apparatus provides the most appropriate biochemical pathways to deal effectively with that environment. For these reasons it is not unusual that we find similar fundamental biochemical processes in species distantly related in evolution. The concept of comparative biochemistry has arisen from the empirical observations that there is a striking biochemical similarity in the basic life processes of most forms of life.

PHS-NIH
Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1961

The terminal pathway by which electrons are routed to oxygen to form water is commonly referred to as the electron transport system. This system in higher animals consists of a series of enzymes which catalyze the stepwise reduction of intermediate electron carriers such as pyridine nucleotides, flavins and cytochromes. These enzymes and carriers of the electron transport system are intimately associated, and they are integrated with other enzymes, the whole of which is bound in a mitochondrial particle in animal cells and in particulate matter in most bacteria.

The particulate unit also contains another series of enzymes (carrying out oxidative phosphorylation) which are related to the electron transport system and are required for the economy of the cell. Energy released during electron transport can be lost as heat or can be trapped in chemical form (oxidative phosphorylation) as adenosine triphosphate (ATP). ATP is required for motility, growth and maintenance of the cell.

It has been extremely difficult to identify individual enzymatic steps in electron transport because many of the enzymes and intermediate carriers could not be dissociated from the particle. Until recently all attempts to dissociate the particle resulted in a simultaneous loss of oxidative phosphorylative activity, consequently these studies, too, were restricted to the intact particle. Particulate systems impose restrictions on the investigator; because he cannot study the isolated enzyme, his conclusions are based upon indirect evidence.

In an effort to circumvent some of these restrictions, an aerobic microorganism with a partially soluble electron transport system was sought. Hydrogenomonas eutropha fulfills several of the desired conditions: (1) the initial portion of the electron transport system is soluble, (2) the terminal portion of the electron transport system is soluble or is associated with very small particulate elements, (3) present evidence indicates this is a simplified system since only one cytochrome is involved and (4) during oxidation of hydrogen tremendous quantities of potential energy are available to be trapped as ATP.

The investigations with H. eutropha have as their objectives the elucidation of the electron transport and the oxidative phosphorylation systems. The first objective is to isolate individual enzymes in the electron transport system and characterize the reactions they catalyze, eventually permitting reconstruction of the entire system with partially purified enzymes. Using this purified system as an energy generating system, it will be possible to study oxidative phosphorylation independently.

The initial steps in electron transport have been found to require flavin mononucleotide (FMN) as a cofactor when diphosphopyridine nucleotide (DPN) is the final acceptor, i.e. $H_2 + DPN \xrightarrow{FMN} DPNH_2$. Partial purification of the enzyme catalyzing the above reaction has been achieved and pre-

liminary evidence suggested that the overall reaction described required the participation of two enzymes. Tentatively this was visualized in the following way: (1) H_2 + FMN ---> $FMNH_2$ and (2) $FMNH_2$ + DPN ----> $DPNH_2$ + FMN. Additional purification of the enzyme and kinetic studies under various conditions will provide additional evidence on this point.

Two characteristics of the enzyme, instability to storage and sensitivity to traces of oxygen, have caused considerable difficulties in these studies. Both of these limitations now have been removed. Conditions have been found which permit storage of the enzyme for six months with little loss in activity, and techniques have been developed and equipment has been designed to minimize exposure of enzyme to air. Thus it is now possible to conduct certain critical experiments heretofore impossible.

In one such series of experiments, it was demonstrated that an artificial electron acceptor, methylene blue, competes with DPN for hydrogen ions. Methylene blue (MB) reduction does not require FMN, whereas FMN is required for DPN reduction. We can conclude that (1) hydrogen is activated by only one enzyme, (2) some common donor supplies hydrogen ions to both methylene blue and DPN, (3) this unknown donor participates before FMN in the proposed pathway. This raises the question of a third step in the reduction of DPN viz (a) H_2 + X ----> XH_2, (b) XH_2 + FMN ----> $FMNH_2$ + X or XH_2 + MB ----> MBH_2 + X and (c) $FMNH_2$ + DPN ----> $DPNH_2$ + FMN. This point is currently under investigation.

Extracts can be heat-treated in such a way as to lose DPN reducing activity without loss of MB reducing activity. If, as this suggests, two or more enzymes are involved in DPN reduction, it might be possible to provide the destroyed enzymes from extracts from cells grown under other conditions. Accordingly, cells were grown with glucose in place of the gas mixture. Extracts from these cells were unable to reduce DPN or MB. When these extracts were used to supplement heat treated regular extracts, methylene blue activity was not stimulated nor did DPN reduction occur. Apparently nothing of the DPN reducing system remained when cells were grown in glucose.

Studies are being made on the second segment of the electron transport system.

K. Biochemical studies on staphylococcal cell walls.

The cell wall is the largest structural unit found in most bacteria. It is the site of all initial host-parasite interactions either with the bacterium as host in phage attack or as a parasite in the case of bacterial infection. The nature of the cell wall and associated membrane is intimately related to the permeability of nutrients and antibiotics into the bacterium. At the present time it seems very probable that an understanding of the

nature of the mechanisms of biosynthesis of their walls is inseparable from an understanding of the nature of the resistance of certain staphylococci to penicillin and other antibiotics.

In spite of the importance of bacterial cell walls only a few laboratories have devoted a full time effort to their study. Recently, Baddiley et al discovered a new class of phosphate-containing wall compounds, the teichoic acids. These teichoic acids occur in most of the bacteria examined and account for 30-60% of the entire dry weight of the cell wall. Very little previous work has been carried out on the teichoic acids of S. aureus.

The present program has the goal of relating the teichoic acid structure of Staphylococcus aureus to the mode of antibiotic resistance and host-parasite interactions mentioned above. Initially a procedure for the complete rupture of Staphylococcus aureus cells followed by purification of the released cell walls from cytoplasmic and nuclear material was established. Five batches of walls were obtained containing 2.3 ± 0.5% Phosphorus. This would give a calculated teichoic acid content of 35% of the cell wall dry weight (assuming 1 ribitol, 1 N-acetyl glucosamine, and ½ alanine per mole of phosphate).

A study of the conditions necessary for extraction of teichoic acid from the walls is in progress. Preliminary evidence indicated that teichoic acid extracted from S. aureus cell walls can be reabsorbed. A study of the nature of this reabsorption is planned. Such a study may provide evidence for the nature of the linkage binding teichoic acid to the remainder of the cell wall.

Our initial studies on the extracted teichoic acid have indicated that this material is heterogeneous. The physical and chemical properties of these different teichoic acids are being studied. It is hoped that ultimately these properties may be related to the immunological specificity of staphylococcal teichoic acid recently demonstrated in another laboratory.

In the future it is hoped that in vitro systems capable of synthesizing either complete cell wall or subunits thereof can be obtained. Such a system would allow a study of biosynthetic mechanisms and antibiotic-sensitive reactions involved in cell wall synthesis. Present studies are being carried out with penicillin-sensitive Staphylococcus aureus. Ultimately, a comparison with penicillin-resistant S. aureus is planned.

Serial No. NIAID 60
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Studies on Cellular and Cell-Free Staphylococcal Penicillinase.

Principal Investigator: Dr. Arthur K. Saz

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961)

Total:	22/12
Professional:	8/12
Other:	14/12

Project Description:

Objectives:

To purify and study penicillinase from penicillin-resistant staphylococci. To study the ecological significance of penicillinase to organisms. To study inhibition and stimulation of penicillinase activity.

Methods employed:

Isolation and purification of cell-free penicillin from staphylococcal cells by standard biochemical techniques. Measurement of penicillinase activity manometrically and iodometrically.

Major Findings:

Since organisms possessing penicillinase activity do not, au naturel, come into contact with penicillin, it seemed likely that penicillinase activity represented a non-specific attack on a fortuitous substrate. In this view, penicillinase activity per se would not be of survival value to the cell. The possibility existed that the penicillinase attacked cyclic peptides in general. Accordingly, the effect of a cyclic hexapeptide and homogramicidin S, a cyclic decapeptide, were tested for growth inhibitory activities against a variety of penicillinase- and non-penicillinase-producing organisms. These compounds in general inhibited the growth of penicillin-sensitive cells and were non-inhibitory to penicillinase-producing cells. Tests for penicillinase-type activity of cell-free extracts derived from the latter cells against the cyclic peptides indicated that the peptides were hydrolyzed. Penicillin-sensitive cyclic peptide-sensitive cells do not hydrolyze the compounds. Accordingly, dinitrophenylation techniques using fluorodinitrobenzene are being employed to determine the precise point(s) of hydrolysis.

Part B. included

A necessary adjunct of the above is purified penicillinase. Thus far, use of column chromatography, electrophoresis and the usual biochemical techniques for enzyme purification have not yielded preparations exhibiting greater than 12-fold purification.

The search for inhibition and stimulation of penicillinase activity has continued. No compounds with greater activity than those reported last year have been found.

Significance to bio-medical research and the program of the Institute:

The work reported above represents the first demonstration of penicillinase activity against compounds structurally unrelated to penicillin. An understanding of the true nature and function of this enzyme is of value to a rational approach to the problem of therapy of penicillin-resistant staphylococcal infections. Inhibition of penicillinase activity is of obvious importance to this program.

Proposed course of project:

1. To purify penicillinase further and to study its properties. 2. To continue the search for an effective inhibitor. Animals studies will be used as indicated.3.To determine locus at which penicillinase attacks the cyclic peptide. 4. To collect various cyclic peptides for further testing as inhibitors of bacterial growth and as substrates for penicillinase.

Part 2 Included: Yes

Serial No. NIAID 60

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Saz, Arthur K., Lowery, Dolores L., and Jackson, Leah J.: Staphylococcal Penicillinase. I. Inhibition and Stimulation of Activity. J. Bact. 82, 298-304, 1961.

Saz, Arthur K.: Introduction to the Panel on Modes of Action of Antibiotics, Conference on Antimicrobial Agents, Oct. 26-28, 1960. Antimicrobial Agents Annual 1960, Plenum Press, New York, 1961, p. 296.

Honors and Awards relating to this project:

Chairman, President"s Fellowship Committee, American Society for Microbiology.

Lecturer (Professor) in Botany, Howard University, Washington, D. C.

Serial No. NIAID 60-A
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Antibiotic Activity of Sea Water

Principal Investigator: Arthur K. Saz

Other Investigators: Dr. Stanley Watson
Woods Hole Oceanographic Institution
Woods Hole, Massachusetts

Cooperating Units: Woods Hole Oceanographic Institution

Man Years (calendar year 1961)
Total: 6/12
Professional: 6/12
Other: 0

Project Description:

Objectives: To investigate the reported antibiotic activity of sea water. To isolate from sea water, compounds active against Gram positive organisms and particularly penicillin-resistant staphylococci.

Methods employed: Treatment of sea water by various methods (extraction, chromatography, electrophoresis) in order to isolate inhibitory factors present. Standard bacteriological techniques.

Major Findings: It is now possible to state that the previously reported antibacterial activity of sea water is not due to the inorganic salt content of the water. Exhaustive dialysis of sea water against either tap or distilled water leaves a preparation inside the bag free of sea water salts. This preparation exhibits undiminished activity vs. all Gram-positive bacteria, including penicillin sensitive and -resistant staphylococci. This experiment settles a controversy which has been raging for 75 years, i.e., is the antibacterial activity due to inorganic salts or to an organic molecule? It is apparent that, since the substance is non-dialyzable, it must possess a molecular weight of at least 10,000. The dialyzed preparation was flash evaporated to 200 x concentration and placed on the Beckman CP Continuous Flow paper electrophoresis apparatus and fractions collected. Activity was manifest at anodal and cathodal effluents but not through the middle or neutral charge area. The reason

Part B. included.

for activity at both poles is presently obscure.

The active compound is specific in that it is lethal for all Gram positive genera tested thus far but inactive versus all Gram negative genera tested. This observation offers an attractive explanation for the old and puzzling observation that greater than 95% of the bacterial genera in the oceans are Gram negative.

From the biological point of view, another interesting observation has been made. It has been noted previously that sea water samples collected from the same area at times showed activity and at other times were inactive. It has now been established that this represents a quantitative rather than a qualitative difference. All waters collected are lethal if concentrated sufficiently and always versus Gram positive forms rather than Gram negative.

Significance to bio-medical research and the program of the Institute: An inhibitor of penicillin-resistant staphylococci would be of prime importance. As a result of the observations that under properly controlled conditions, Gram negative organisms are not killed by sea water, raises anew the problem of the advisability of running either treated or untreated sewage into oceans.

Proposed course of project: A cooperative arrangement has been worked out with Woods Hole Oceanographic Institution for continuation of the problem both here at the NIH and it is hoped at the Oceanographic Institituion. Isolation of the substance(s) will be attempted. To get some idea of the type of large molecule involved, the concentrated activity will be treated with various degradative enzymes such as lipases, proteinases, amylases, etc., to determine whether the activity is altered. Further, an attempt will be made to collect and concentrate large amounts of sea water and to determine the mode of action of the inhibitory compounds. An attractive possibility since Gram positive forms are so clearly differentiated from Gram negative by the substance, is that the attack is upon cell wall metabolism.

Part 2 Included: Yes

Serial No. NIAID 60-A

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Honors and Awards relating to this project:

Appointed Research Associate at the Woods Hole Oceanographic Institution, Woods Hole, Mass.

Serial No. NIAID 61
1. Infectious Diseases
2. Medical & Physiological
Bacteriology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1961.

Part A.

Project Title: The electron transport system in an autotrophic hydrogenomonad.

Principal Investigator: Dr. Roy Repaske

Other Investigators: None

Cooperating Units: None

Man years (calendar year 1961)

Total:	22/12
Professional:	12/12
Other:	10/12

Project Description:

Objectives: This research project has as its object the identification of individual enzymes and intermediary cofactors which are operative in the electron transport system of Hydrogenomonas eutropha. The findings may be applicable in part to mitochondrial systems and provide important clues about the electron transport sequence in these particulate structures. The long range plan will include an investigation of the energy yielding reactions coupled to electron transport.

Methods employed: Growth of bacteria in large quantities is required to provide source material for the identification, isolation and purification of electron transport enzymes. As difficulties are encountered (difficulties inherent in all scaling-up operations for larger cultures), necessary changes in the design of the equipment are being made. Enzymes and assay systems sensitive to traces of oxygen contamination require special techniques which must be devised for each new step in the purification and assay of the enzymes. Enzyme purification is being achieved by use of techniques such as selective protein precipitation, gel adsorption and elution, solvent precipitation and chromatography.

Major Findings: The role of riboflavin phosphate (FMN) in diphosphopyridine nucleotide (DPN) reduction by hydrogen has been substantiated by showing direct enzymatic reduction of FMN by hydrogen. Indirect evidence

Part B. included.

supporting the concept of a two step reaction for DPN reduction was obtained by the simultaneous use of two acceptors; these results suggested reducing equivalents were provided to each of the acceptors by a common intermediate. The above system has been purified 9 fold and this more unstable preparation has been successfully stabilized by the development of special techniques for handling and storing the enzyme preparation. In addition, it was discovered that certain cations are deleterious to this enzyme.

Significance to bio-medical research and the program of the Institute: The oxidation of nutrients by oxygen, a biochemical process shared by all oxygen requiring organisms, simultaneously yields energy in the form of heat in an uncoupled system or in the chemical form of ATP if phosphorylation is coupled to oxidation. ATP is required for the synthesis of new cell material. Thus only in a coupled system does oxidation provide appropriate "chemical energy" needed for cell growth and repair.

Hydrogenomonads are unique organisms which obtain energy for all of their life processes through the oxidation of hydrogen gas in the presence of oxygen; in contrast, animal cells and most bacteria oxidize organic substances for energy. Although the immediate substance oxidized by hydrogenomonads is different, biochemical studies in the past have demonstrated that a common denominator exists for basic biochemical processes in all organisms. This well documented concept of comparative biochemistry is the rationale for investigating a common biochemical reaction in any organism which yields an enzyme preparation most amenable to study.

In most organisms studied, the electron transport system which mediates oxidation is associated with particulate elements of the cell and many of the enzymes involved can not be solubilized and purified as soluble enzymes. The same difficulty exists in regard to some of the cytochromes. Hydrogenomonas eutropha on the other hand has a partially soluble electron transport system and preliminary studies indicate only one cytochrome participates in these reactions. For these reasons it is felt that some limitations associated with other systems may not exist in the H. eutropha system.

Proposed course of project: Additional purification of the enzyme involved with hydrogen activation and DPN is in progress. When purification has achieved the separation of these enzymes from subsequent enzymes in the sequence, a second system, the DPNH oxidase system, will be analyzed. The role of the native cytochrome c and the nature of terminal oxidase will be investigated. The separation of individual enzymes of this sequence will be continued.

Part B Included: Yes

Serial No. NIAID 61

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Wittenberg, C. L. and Repaske, R.: Studies on hydrogen oxidation in cell-free extracts of *Hydrogenomonas eutropha*. Biochim. et Biophys. Acta, 47, 542-552, 1961.

Repaske, R.: The electron transport system of *Hydrogenomonas eutropha*. I. DPN reduction by hydrogen. J. Biol. Chem., In press.

Repaske, R.: Nutritional requirements for *Hydrogenomonas eutropha*. J. Bacteriol., In press.

Honors and awards relating to this project:

Elected Secretary of the Division of the American Society for Microbiology.

Invited to Vth International Biochemical Congress, Moscow (Aug. 9-16, 1961) and presented a paper, entitled: "Enzymatic reduction of DPN by hydrogen gas."

Appointed a consultant for the Martin Company, Baltimore, in regard to an aspect of their space research program.

Serial No. NIAID 62
1. Infectious Diseases
2. Medical & Physiological
Bacteriology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1961.

Part A.

Project Title: Biochemical studies on staphylococcal cell walls.

Principal Investigator: Dr. Eskin Huff

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961)
Total: 25/12
Professional: 12/12
Other: 13/12

Project Description:

Objectives: The general objective of this project is an understanding of the chemical mechanisms involved in the synthesis of Staphylococcal cell walls and the manner in which penicillin affects this synthesis. It is hoped that a study of differences in the chemical composition and synthesis of cell walls by penicillin-sensitive and -resistant Staphylococci will throw some light on the mechanism of antibiotic resistance in these organisms.

Specifically an attempt is being made to characterize chemically the organic phosphate-containing compounds occurring in the cell walls of Staphylococcal species.

Methods employed: A strain of Staphylococcus aureus has been grown in 30 liter batches on trypticase-soy-broth, harvested in a Servall continuous flow centrifuge, and washed at low speed in the International PR-2 centrifuge followed by sedimentation in the swinging bucket head of the Spinco ultracentrifuge at 45,000 x g. Cells are stored at -20 C at a concentration of 10^{11} viable counts per ml. As soon as the cell suspension is thawed it is shaken with glass beads for five 20 second periods in a Nossal shaker. The suspension is cooled in an ice bath between shakes. Cell walls prepared in this manner have been washed 9 times by suspension in distilled water followed by sedimentation at 38,000 x g. Treatment of the walls with 10% trichloracetic acid has been used to extract the organic phosphate components associated with the cell wall.

A procedure for determining the relative amounts of whole cells and cell walls has been developed. A one ml. aliquot of a suspension of cells and cell walls is placed in a 5 x 68 mm lusteroid tube in a special holder and spun for 10 minutes at 45,000 x g in a Spinco swinging bucket head. Cell walls pack as a distinct layer above the whole cells. The relative lengths of the packed zones are taken as a measure of the relative amounts of cells and cell walls.

Major Findings: Studies on changes in optical density, viable count and cell wall and whole cell packed volume at 45,000 x g have led to the development of a procedure for the complete breakage of cells with minimal further breakdown of the cell walls. A study of the release of phosphate into the supernatant with washing indicated that little phosphate was removed after 9 washings. In 4 different wall preparations a gradual decrease in the phosphorus content from 2.6 to 1.9% of the dry weight was observed. This decrease may be related to the increased care with which the walls were washed.

When cell walls were extracted for 24 hours with 10% trichloracetic acid at 0°C as used by Baddiley for extraction of polyribitol compounds from several organisms, only 20 to 25% of the total phosphate of the wall was liberated. Time studies revealed that phosphate was still being liberated. Time studies revealed that phosphate was still being liberated from the walls after 24 hours at an undiminished rate. Thus twice as much phosphate could be extracted in 48 hours as in 24 hours. In order to hasten the extraction the temperature was raised to 37°C. This resulted in a 50-fold increase in the rate of extraction of the phosphate containing compounds. Thus 75% of the phosphate could be removed by a single 4 hour extraction and all but 4% by 24 hours. After 24 hours of extraction 62% of the dry weight of the cell walls had been solubilized.

Dialysis and ultrafiltration studies of a 4 hour (10% TCA; 37°) extract of walls indicated that more than one molecular weight fraction is present. One is ultrafilterable and partially dialyzable and the other is not. In accord with these results is the fact that the organic phosphate of a 2 hour extract can be separated into two major fractions upon chromatography on Sephadex.

Significance to bio-medical research and the program of the Institute: Since the initial report in 1958 by Baddiley and coworkers of the presence of polymers of ribitol phosphate and glycerol phosphate in the walls of Bacillus subtilis, Lactobacillus casei, and Staphylococcus aureus, the only published study of these compounds in S. aureus is the recent report (Aug. '61) by Mandelstam, Strominger, Sanderson, and Juergens. These authors have corroborated the presence of D-alanine and N-acetyl-glucosamine in S. aureus polyribitol phosphate originally reported by Baddiley. They have also presented convincing evidence that the polyribitol phosphate polymer containing N-acetyl glucosamine is responsible for determining the antigenic specificy of the strain of S. aureus studied.

Very little is known about the relative amount of polyribitol phosphate and muramic peptide in whole cell walls. It is not known how these two polymers are linked. The molecular weight of S. aureus polyribitol phosphate is not known. Little or nothing is known about the manner in which this polymer of ribitol phosphate or whole cell wall is made biosynthetically. Although it has been demonstrated that penicillin stops the synthesis of cell wall peptides without an affect on the synthesis of intracellular protein, it is not known what affect penicillin has on the synthesis of cell wall polyribitol phosphate.

A study of the biosynthesis and chemistry of the phosphate compounds of Staphylococcal cell walls appears to be a reasonable approach to an understanding of the manner in which cell wall synthesis is affected by penicillin.

Proposed course of Project: Attempts to prepare large batches of the two major phosphate containing components of S. aureus cell wall are being pursued. A study of the structure of these compounds is planned. Further, attempts to determine whether these two components exist as such in walls or are generated as an artifact of extraction are planned. Schemes for determining the mode of attachment of the polyribitol phosphate to cell wall are being considered. In the future, a study of the pathway of biosynthesis of the cell wall is planned. These questions are being pursued in the penicillin sensitive strain of S. aureus now under study. Ultimately, a comparison will be made with penicillin-resistant S. aureus.

Part B Included: Yes

Serial No. NIAID 62

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Huff, Eskin: The Metabolism of 1,2-Propanediol. Biochim. et Biophys. Acta, 48, 506-517, 1961.

Honors and Awards relating to this project:

None

Serial No. NIAID 63
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Non-specific Immunity Associated with Serum Siderophilin and the Iron Metabolism of both Pathogen and Host.

Principal Investigator: Dr. Arthur L. Schade

Other Investigators: Dr. Lathrop E. Roberts
Dr. Austin Shug

Cooperating Units: None

Man Years (calendar year 1961)

Total:	25/12
Professional:	15/12
Other:	10/12

Project Description

Objectives:

To investigate the contribution of blood serum siderophilin to the natural resistance of the host; to investigate the iron requirements of selected pathogens and the effect of the iron-chelating siderophilin and conalbumin on *in vivo* iron accumulation, growth, and metabolism of these pathogens; to investigate the factors leading to changes in concentration of both serum iron and siderophilin in circulating blood as a result of infection and other abnormal conditions, and the consequence of such changes on resistance to infection, iron absorption by the host, and anemia.

Methods Employed:

Bacterial and cultural procedures in specially deironized media; spectrophotometric, chromatographic, and radioisotopic methods; metabolic and enzymatic analytical procedures; and methods particularly suited to estimation of iron chelated either to siderophilin or conalbumin and to their relative unsaturations.

Part B. included.

Major Findings:

Previous findings have established that some bacteria will not grow in serum unless the contained siderophilin is saturated with iron, while the growth rate of many others in serum is a function of the percentage iron-saturation of the siderophilin. We have, for our more intensive research investigations, restricted ourselves to Staphylococcus albus, whose growth in normal serum is negligible in the absence of excess iron, and to Staphylococcus aureus, whose growth rate in serum reflects the amount of bound serum iron compared with its total iron-binding capacity.

Proceeding from our preliminary studies on the metabolism of Staph. aureus cells grown in medium non-restrictive with respect to iron and those grown in a medium whose concentration of free ionic iron was severely limited by the presence of either siderophilin or conalbumin at low iron saturation (5%) we have observed the following additional facts:

1. Growth characteristics of Staphylococcus aureus cells in trypticase-egg white media of high and low iron content were as follows: the lag period was of 5 hours duration for high iron cells and 8 hours for low iron cells; both high and low iron cells reached the stationary phase of growth in about 15 hours at 37°C. Radioactive Fe^{59} tracer experiments showed that high iron cells accumulated increasing amounts of iron, while in low iron cells the amount of iron remained constant throughout the growth period. Manometric experiments employing these high and low iron cells established that the previously reported metabolic differences of such cells is independent of the physiological age of the cells. (See 1960 Project Report NIAID #63). Cell-free extracts of high and low iron cells were made by sonic disintegration. After high-speed differential centrifugation, DPNH oxidase activity was found in the particulate fraction; while DPNH dehydrogenase activity remained in the soluble fraction. These enzymatic activities were determined to be greater in the extracts of the iron-rich cells but precise quantitative evaluation remains to be done.

2. A search for nucleotide cell-wall precursors in iron-deficient Staph. aureus cell walls was undertaken. This investigation was suggested by the work of Park, Strominger, and others on the effect of penicillin on Staph. aureus cell walls and by our observation that iron-deficient cells suspended either in nutrient or non-nutrient buffer solutions underwent spontaneous lysis more rapidly than iron-rich cells. Trichloracetic acid extracts of both iron-rich and iron-deficient cells, following chromatographic absorption and elution, were examined on paper chromatograms with u.v. light for respective nucleotide patterns. No differences were detected between the extracts from the two types of cells.

3. A study of the metabolism of glucose by iron-rich and iron-deficient Staph. aureus cells was initiated utilizing glucose-1-C^{14}, glucose 6-C^{14} as well as uniformly labeled glucose as substrates. Among

the results of significance obtained, we have found: (a) that although both types of cells metabolize all the glucose offered them, the iron-rich cells consume enough oxygen for complete oxidation of 50 to 60% of the total glucose present while iron-deficient cells consume only enough oxygen for approximately 15% of the glucose; (b) while iron-rich cells yield radioactive CO_2 from 6-C^{14}-glucose in an amount equal to one-half that produced from 1-C^{14}-glucose, iron deficient cells produce no radioactive CO_2 from 6-C^{14}-glucose in contrast to the considerable $C^{14}O_2$ production from 1-C^{14}-glucose. Both these results attest to an oxidative deficiency of iron-deficient cells and, from the latter result, it is evident that such cells lack the ability to oxidize or decarboxylate the moiety of the metabolized glucose containing the 6-C^{14}.

Analyses of the radioactivities of the cells and their supernatants from these experiments of radioactive glucose metabolism by iron-rich and iron-deficient cells of Staph. aureus revealed an apparent defect in the ability of iron-deficient cells to incorporate carbon into cell material compared to iron-rich cells. For example, iron-rich cells incorporated 65% of the C^{14} from 1-C^{14}-glucose, not accounted for by respired $C^{14}O_2$, leaving the remaining 35% as other end products in the supernatant. Iron-deficient cells, in the same medium, incorporated but 21% of this C^{14}, leaving 79% in the supernatant. The corresponding values for 6-C^{14}-glucose as the substrate were: 56% C^{14} in the iron-rich cells and 44% C^{14} in their supernatant compared to 15% C^{14} in the iron-deficient cells and 85% C^{14} in their supernatant.

Significance to the bio-medical research and the program of the Institute:

The study of the naturally occurring iron-chelating serum protein, siderophilin, contributes to our understanding of the non-specific immunity mechanism available to humans for protection against microbial infection. Investigation of the qualitative and quantitative effects of siderophilin on the growth and metabolism of the pathogen Staphylococcus aureus will provide useful information on the pathogenicity of this bacterium especially under conditions closely approximating in vivo conditions.

Proposed Course of Project:

We plan to continue our investigation of the growth, metabolism, and enzymatic capabilities of S. aureus when grown under conditions approximating the in vivo state with especial regard to the effect of different serum iron-saturation levels on such characteristics. Emphasis will also be placed upon the biochemical mechanisms by which iron controls the overall metabolism of the organism. Depending upon the availability of qualified personnel, we should like to investigate both qualitatively and quantitatively the production of staphylotoxins by Staphylococcus aureus grown in serum at high and low iron levels. Additional biological features such as hemolysis, effectiveness of phagocytosis, and possible differences in response to antibiotics of high and low iron-containing cells should be studied.

Part B Included: Yes

Serial No. NIAID 63

PHS-NIH
Individual Project Report
Calendar Year 1961.

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Schade, Arthur L.: The microbiological activity of siderophilin. Protides of the Biological Fluids, Proceedings of the 8th Colloquium, Bruges 1960. Elsevier Pub. Co., Amsterdam 1961, pp. 261-263.

Rifkind, David, Kravetz, Howard M., Knight, Vernon and Schade, Arthur L.: Urinary excretion of iron-binding protein in the nephrotic syndrome. New England J. Med., 265, 115-118, 1961.

Serial No. NIAID 63-A
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Biochemistry of the acquisition of iron by mammalian tissues as mediated by the iron-binding protein, siderophilin.

Principal Investigator: Dr. Arthur L. Schade

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961)
Total: 27/12
Professional: 7/12
Other: 20/12

Project Description:

Objectives: To investigate the mechanism by which mammalian tissues acquire from serum siderophilin the iron required for normal growth and function and to study the effect of physiological conditions upon this exchange.

Methods Employed: Hematological procedures, radioactive tracer techniques, manometric techniques and immunologic analyses.

Major Findings: Siderophilin is the specific iron-binding protein component of the circulatory system fluids, serum and lymph, and has already been shown to possess three biological functions: iron transport in the body, non-specific immunity against iron dependent pathogenic bacteria, and inhibition of the production of lipid peroxide in the circulating blood. All three functions are ascribable to the specific iron-chelating capability of the protein. To study and exploit the properties of siderophilin for clinical use in infectious and constitutional diseases it is necessary to possess isolated siderophilin in native form and in quantity. During this year, we have continued our search for a simple, reproducible method to test for the "undenatured" and physiologically fully active preparation of isolated siderophilin. We have succeeded in the development of a liver mince-iron uptake method which, when used in conjunction with our reticulocyte iron uptake procedure (See 1960 Project Report NIAID #63-B), establishes the physiological suitability of the isolated siderophilin for laboratory investigation and for clinical use. A comprehensive discussion of the several siderophilin analytical methods developed by this laboratory provided the substance of a paper given in Moscow

Part B. included.

at the International Biochemical Congress while a detailed presentation of the new liver-mince test method formed the basis of a paper presented to the European Hematology Congress at Vienna.

Utilizing both our reticulocyte and liver-mince iron-uptake tests for evaluation of the adequacy of several isolated siderophilins, we concluded that siderophilin isolated through the use of Rivanol as precipitant is physiologically "denatured". Our evidence suggests that satisfactory siderophilin can be obtained by differential salt precipitation and column chromatographic procedures.

Studies with reticulocytes, liver-mince, and cells of Staph. aureus as iron absorbing agents from an iron-siderophilin source have established that reticulocytes and bone marrow possess a specific mechanism for the accumulation of iron from this protein chelate referable to the specificity of the protein molecule itself. Liver-mince and staphylococcal cells depend for their iron on the natural dissociation of the metal-protein complex itself and take up the ionic iron thus made available.

Significance to bio-medical research and the program of the Institute:

The conditions of availability of serum iron to host tissues and the mechanism of its transfer from siderophilin are of significance to host-parasite relationships obtaining in infections. The requirement for having isolated, physiologically active siderophilin available in quantity for clinical investigation of pathological states involving iron metabolism of either infecting agent and/or host is evident and becoming increasingly pressing. Knowledge of the chemistry of the iron-protein itself is meager and must be augmented to assure application of this naturally-occurring iron chelator in appropriate clinical situations.

Proposed course of project:

We propose to continue our investigation of the chemistry of the iron-siderophilin complex; to extend our knowledge of the manner in which various cells and tissues satisfy their iron requirements through the intervention of iron-siderophilin; and to make such applications of our test methods and to improve them so as to assure the production and availability of adequate quantities of "undenatured" siderophilin for use by clinicians in their investigations.

Part B Included: Yes

Serial No. NIAID 63-A

PHS-NIH
Individual Project Report
Calendar Year 1961.

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Woodworth, Robert C., and Schade, Arthur L.: Immunological Precipitin Titrations Based on Radioactive Tagging of the Iron Naturally Chelated by the Proteins Siderophilin and Conalbumin. Biochim. Biophys. Acta, 47, 403-405, 1961.

Honors and Awards related to this project:

Invited to participate in the 5th International Congress of Biochemistry, Moscow, Aug. 10-16 and to give a paper entitled: "Siderophilin Regulation of Iron Uptake by *Staphylococcus aureus*, Rabbit Liver, Bone Marrow, and Reticulocytes."

Invited to participate in the 8th Congress of the European Society of Hematology, Vienna, Aug. 28-Sept. 2, and to give a paper entitled "A New Physiological Method for Evaluating the Integrity of Isolated Siderophilins." Invited to lead a Discussion in a Round Table devoted to "Iron Metabolism."

Invited to prepare a review article on "Siderophilin" for the journal, "Vox Sanguinis".

Serial No. NIAID 63-B
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1961.

Part A.

Project Title: Coliform and Pseudomonas Urinary Tract Infections Refractory to Antibiotic Drug Therapy.

Principal Investigator: Dr. Arthur L. Schade

Other Investigators: Dr. William W. S. Butler

Cooperating Units: None

Man Years (calendar year 1961):
Total: 3/12
Professional: 1/12
Other: 2/12

Project Description:

Objectives: To reinvestigate the isolation and development of bacteriophages specific for antibiotic-resistant strains of Escherichia coli, Aerobacter aerogenes, Proteus vulgaris, and Pseudomonas aeruginosa; to produce phage lysates of high titer and virulence; to introduce such lysates into the urinary tract including the kidney pelvis; and to follow the course of the infection and its treatment with careful medical and urological supervision. To investigate the use of lysozyme alone and in combination with ethylenediaminetetraacetic acid solutions in the urinary tract for treatment of Pseudomonas aeruginosa infections and to assess the effect of bacteriophage lysates plus appropriate lysozyme mixtures on these and coliform infections.

Methods Employed: Standard bacteriological and bacteriophage procedures. Professional urological methods for therapy of urinary tract infections with patient care and follow-up.

Major Findings: Based upon a previous experience with the successful use of specific Proteus bacteriophage lysate in the treatment of a urinary tract infection, we instituted a course of treatment recently for a patient suffering from a long standing (more than 5 years) chronic E. coli urinary tract infection involving 2 bacterial strains. Following introduction into the pelvis of each kidney of 10 ml of high titer lysate specific for the 2 E. coli strains, the patient over the past month has voided only sterile urine and has improved generally in health.

Significance to the bio-medical research and the program of the Institute: The incidence of refractory Pseudomonas and the Coliform urinary tract infections is considerable and represents a significant proportion of the difficultly manageable medical problems facing the urologist today.

Proposed Course of Project: We propose to undertake the necessary steps to realize the objectives summarized above.

Serial No. NIAID 64
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Detoxification of Potential Tuberculostatic, Fungistatic, Parasiticidal and Viricidal Agents.

Principal Investigator: Dr. Benjamin Prescott

Other Investigators: None

Cooperating Units: None

Man Years (Calendar Year 1961)
Total: 13/12
Professional: 3/12
Other: 10/12

Project Description:

Objectives: Further studies on toxicity and detoxification of streptomycin (SM), isoniazid (INH), mixtures of streptomycin-isoniazid, cycloserine, kanamycin and neomycin. To find means of reducing further the toxicity of these drugs without interference with bacteriostatic activity. In addition, to find means of reducing the toxicity of widely used fungistatic, parasiticidal and viricidal drugs.

Methods Employed: The effect of various vitamins, vitamin analogs, amino acids, keto acids as well as glycerine, glycerol formal and aromatic sulfonic acids on the toxicity of SM and/or INH, neomycin, kanamycin, and cycloserine was measured by (a) the reduction in toxic effects and survival time of two strains of mice following concomitant subcutaneous or oral administration of sublethal and lethal amounts of the drugs with each adjuvant in both single and multiple administration, (b) blood plasma levels of isoniazid as measured by the "pyridyl" method, (c) the *in vitro* bacteriostatic action of the drug-adjuvant combination. With the parasiticides, the agents were given simultaneously with the drugs to infected animals.

Major Findings:

1. With streptomycin-isoniazid mixtures (SM-INH). Further studies have included the use of appropriate amounts of glycine and

Part B.included.

sodium glucuronate. At levels of 50 mg of each adjuvant per 20 gram mouse, 37 of 40 (93%) DBA mice tolerated the administration of 15 mg SM plus 8 mg INH which is 100% lethal per se. The use of these detoxifying agents increased mouse tolerance of SM-INH mixtures up to three times the lethal dose and permitted repeated and longer continued treatment. The combinations gave sustained blood levels and the detoxifying agents were without effect on the chemotherapeutic activity of the drugs.

2. With aromatic sulfonic acids: Six aromatic sulfonic acids were tested as adjuvants with mixtures of SM-INH. Of the six tested with various mixtures of the two therapeutic agents in DBA mice, 6-thymolsulfonic acid showed excellent detoxifying action. 15 mg SM plus 8 mg INH/20 gram mouse (3 x lethal dose) was tolerated by 85% of the mice when administered subcutaneously with 50 mg of 6-thymolsulfonic acid. Mice also tolerated repeated daily administration of the mixture.

3. With cycloserine: Since this tuberculostatic agent has also been found effective against Nocardia infections, further means of detoxifying the drug were undertaken. Six alpha keto acids were tested as adjuvants. Of the six, both sodium pyruvate and alpha-ketoglutaric acid (neutralized) were effective in doses of 25 mg in permitting survival of mice given toxic doses ([illegible] mg/20 gram mouse) of the chemotherapeutic drug in both single and repeated doses.

4. With Miracil-D: Various complex monocarboxylic acid salts of the drug showed excellent detoxifying action. 30 mg of Miracil D/20 gram mouse (6 x lethal dose) was tolerated by 100% of the mice when administered by gavage simultaneously with either 50 mg of p-aminobenzoic, benzoic or p-aminosalicylic acids. In addition, an iodide complex of Miracil D was tested orally for toxicity in DBA mice. 20 gram mice will tolerate only 5 mg (250 mg/kg) of Miracil D orally for 5 days. With the iodide complex, mice survived 20 mg doses.

5. Detoxification of kanamycin: Since calcium pantothenate and nicotinic acid proved effective in permitting mouse survival when administered simultaneously with a toxic dose of SM, a comprehensive study was carried out in DBA mice on the effect of each vitamin on the toxicity of kanamycin with and without vitamin. The results on mice treated with 25 mg kanamycin daily for 30 days showed that 30 of 40 animals (75%) survived while with a 14 mg dose of calcium pantothenate as adjuvant 39 of 40 (98%) of the mice tolerated the dose of drug. With 25 mg of nicotinic acid (neutralized) 37 of 40 mice (93% tolerated 30 subcutaneous administrations of the kanamycin.

6. Detoxification of Neomycin: With 35% glycerol-formal as solvent, a lethal 10 mg dose of neomycin sulfate was tolerated by [illegible] of a group of 70 DBA mice and with [illegible] mg Neomycin sulfate dissolved in [illegible] glycerol formal 72 of 80 mice (90%) survived.

7. With alpha keto acids: Of six alpha-keto acids, alpha-

ketoglutaric acid (neutralized) and sodium pyruvate were effective in doses of 10 and 25 mg respectively in permitting survival of mice when given simultaneously with a lethal 10 mg dose of neomycin sulfate.

Significance to bio-medical research and the program of the Institute:

Increase of tolerance to any of the above toxic chemotherapeutic drugs whether by reducing toxic manifestations or by permitting larger dosage without loss in therapeutic activity should result in more effective therapy in antimicrobial infections.

Proposed course of the project:

In addition to the indicated procedures with the above studies, increased emphasis will be placed on *in vivo* tests in experimentally infected animals.

Part B Included: Yes

Serial No. NIAID 64

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Prescott, Benjamin and Stone, H. J.: Means of increasing the tolerated dose of streptomycin in man - Steroids. Antibiotics and Chemotherapy (Accepted for publication)

Honors and Awards relating to this project:

Invited and participated in "Symposium uber experimentelle und klinische Pharmakologie der Antibiotica" held in Aachen, Germany May 18-19 (1961). Presented a paper on the "Detoxification of streptomycin and kanamycin."

Serial No. NIAID 64 - A
1. Infectious Diseases
2. Medical & Physiological
Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Potential Fungistatic, Parasiticidal, Leprocidal and Viricidal Agents.

Principal Investigator: Dr. Benjamin Prescott

Other Investigators: None

Cooperating Units:

Section on Mycology, LID-NIAID
Dr. Chester W. Emmons - Serial No.NIAID 76-B

Section on Chemotherapy, LPC-NIAID
Dr. George Luttermoser

Lab. of Pharmacology & Toxicology, NIAMD
Dr. Y. T. Chang

Laboratory of Virology and Rickettsiology
Division of Biologics Standards
Dr. Chen Pien Li

Man Years (calendar year 1961)
Total: 13/12
Professional: 3/12
Other: 10/12

Project Description:

Objectives: Synthesis of new potential non-toxic chemotherapeutic agents against fungal, parasitic, leprotic and viral infections.

Methods Employed:

Antifungal agents: A series of 2,3 dichloronaphthoquinone derivatives were synthesized, condensing this compound with numerous aliphatic and aromatic amines in 95% ethyl alcohol. The resulting brightly colored derivatives were recrystallized from various organic solvents (alcohol, dioxane, benzene) and analysed for carbon, hydrogen and nitrogen. In addition, steroid derivatives of 4,4'-diaminodiphenyl-sulfone, thymol thiosemicarbazones, a series of 40 carboxymethyltrimethylammonium chloride hydrazones, 40 oxalyldihydrazones and guanylhydrazones were synthesized. They are being tested for antifungal activity by Dr. Chester Emmons. Studies in DBA mice showed that toxicity of these compounds was minimal.

Parasiticides: Complex salts of tetracycline and Miracil D with iodine and sulfonic acids were prepared. These complex salts show an acute toxicity much lower than that of the corresponding hydrochlorides. In mice, the intraperitoneal LD_{50} of the new complex salts is about 1 g/kg for the tetracycline complexes of phenolsulfonic, camphorsulfonic, pyridine-3-sulfonic and metanilic acids; and 2 g/kg for the Miracil D-iodide complex as compared with 200 mg/kg for the tetracycline hydrochloride and 500 mg/kg for the Miracil D-hydrochloride. In addition, several new piperazine derivatives were synthesized by coupling piperazines with methyl, ethyl and phenyl chlorothioformates in the presence of sodium bicarbonate. These and several antihistamines and numerous iodine compounds synthesized are being tested by Dr. George Luttermoser against parasitic infections in animals in Lebanon.

Antiviral agents: Several thiosemicarbazones of long chain fatty amines are being tested by Dr. C. P. Li against viral infections. These same compounds and several hydrazones are also being studied by Dr. Y. T. Chang for possible chemotherapeutic activity against rat leprosy.

Major Findings:

Several of the iodine complexes and tetracycline derivatives demonstrated considerable *in vitro* activity against parasites. Of the series of crystalline steroid derivatives of 4,4'-diaminodiphenylsulfone synthesized, none was as toxic as the original sulfone.

Significance to bio-medical research and the program of the Institute:

Because of high tolerance and effectiveness these compounds may be useful as potential chemotherapeutic drugs.

Proposed Course of Project:

Tests for usefulness of these compounds are being performed to determine their effectiveness in experimental parasitic, fungal and viral infections in laboratory animals. Numerous derivatives of the thiosemicarbazones and hydrazones will be tested against rat leprosy and viral infections in mice. Also, about 300 compounds have been sent to the Cancer Chemotherapy Screening Program. Several salts of thioxanthanones which were effective in initial antitumor tests in mice are being further evaluated.

Serial No. NIAID 64-B
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Isolation, purification and characterization of antibacterial and antiviral substances from mollusks.

Principal Investigator: Dr. Benjamin Prescott

Other Investigators: None

Cooperating Units: Laboratory of Virology & Rickettsiology
Div. of Biologics Standards
Dr. Chen Pien Li

Man Years (calendar year 1961)

Total:	16/12
Professional:	6/12
Other:	10/12

Project Description:

Objectives:

A search for antimicrobial agents in certain mollusks. Purification and characterization of these substances and tests for antibacterial activity *in vitro* and *in vivo* against experimental *Staphylococcus aureus* and streptococcal infections in mice. Procedures for assay of antiviral activity of the fractions isolated were performed in tissue culture and/or in mice by Dr. C. P. Li.

Methods employed:

Antibacterial fractions are isolated from abalone juice by ion-exchange chromatography. The material was purified by fractional precipitation with cold ethyl alcohol or by $(NH_4)_2SO_4$ precipitation.

Major Findings:

The most active material is present in the first eluate. Extraction of the meat from the mollusks with 25% acetic acid gave a frac-

Part B. included.

tion that was high in antiviral activity. The isolate when lyophilized is a whitish powder, readily soluble in water. It is non-dialyzable and stable at a temperature of 95-100° C for 30 minutes. The isoelectric point of the purified material is pH 4.4. Ultracentrifuge experiments indicate it consists of a single component with a molecular weight of 5000 to 10,000. Hydrolysis of samples by gentle boiling with 6 N-HCl at 130° C for 20 hours shows 18 amino acids present in every hydrolysate when chromatographed on Whatman #3 paper. Fractions with similar characteristics and almost identical antibacterial activity were also isolated by the same technique from juice squeezed from fresh oysters (*Ostrea virginica*) and water extracts of sea snails (*Tegella gallina*). It is evident from the ion-exchange chromatography experiments that there are two groups of active fractions present in the mollusks tested. Group A representing the early eluates showed high antibacterial activity against *Staphylococcus aureus* and other bacteria. This group showed no antiviral activity. Group B representing a final M NaCl wash from the column contained no antibacterial activity. However, it showed definite inhibition against influenza type A virus, Japanese 305 strain and polyoma virus (designated as 5019ME9).

Significance to bio-medical research and the program of the Institute:

Chemical results will aid in identification of the components and enable duplication of the effective agents. These agents might constitute another class of factors concerned with defensive mechanisms by exerting a lethal action on invading organisms. A need for effective antiviral agents is still indicated.

Proposed Course of Project:

Fractionation of juice and meat from various molluks (Queen conch, clams, quahogs, snails) is being continued. This project receives increased emphasis this year because of larger amounts of material available for testing against bacterial and viral infections in animals.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Li, C. P., Prescott, B., and Jahnes, W. G.: Antiviral activity of a fraction of abalone juice. Proc. Soc. Exp. Biol. and Med. (accepted for publication)

Honors and Awards relating to this project:

Invited to participate and present a paper on the "Isolation and characterization of antimicrobial substances from mollusks" at the 2nd Congresso Latinamericano y I Nacional de Microbiologica to be held in San Jose, Costa Rica, Dec. 10-17 (1961).

Participated in the 45th Annual Meeting of the Federation of American Societies for Experimental Biology, April 1961; co-author of paper "Antiviral Activity of Abalone Juice" which was presented at these meetings.

Serial No. NIAID 65
1. Infectious Diseases
2. Medical & Physiological
Bacteriology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: The biology of group A streptococci.

Principal Investigators: Dr. Roger M. Cole, Dr. Jerome J. Hahn

Other Investigators: Miss Edythe J. Rose, Miss Alba E. Colon

Cooperating Units: None

Man Years (calendar year 1961)
Total:
Professional:
Other:

Project Description:

Objectives: To study the biology of Group A streptococci, their surface antigens and antibodies, in order to comprehend better the interrelationships of growth, cell wall formation and structure, virulence, pathogenesis and resistance to infection -- not only among streptococci or gram-positive cocci, but also among bacteria in general.

Methods Employed: There has been no substantial change in the direction of our research, and the methods used were described in some detail last year (1960; Serial No. NIAID 65-A). A principal current tool is growth of the streptococcus in fluorescein-labelled homologous antiserum or globulin, followed by various manipulations such as antibody removal, antibody replacement, addition of antibiotics, addition of phagocytes, addition of streptococcal extracts, etc. Standard methods of bacteriophage study are employed, but modifications particularly suited to streptococci are a primary goal in our beginning research in this area.

Major Findings:

(a) Use of fluorescent antibody in following cell wall replication of streptococcus. Fluorescein-labelled homologous antibody (either group- or type-specific) has been used as the culture medium for Group A streptococci. In such a medium, there is continuous fluorescent labelling of newly-formed coccal cell wall by binding of the antibody with the re-

Part B. included.

spective cell wall antigen, "C" polysaccharide (group-specific) or "M" protein (type-specific). After removal from the medium of free labelled antibody by specific precipitation, or its inhibition by addition of excess unlabelled antibody, coccal growth continues and newly-formed portions of the cell wall are now non-fluorescent. Complementary labelling is obtained by a reverse technique of initial growth in unlabelled antibody, followed by antibody removal, continued growth, and subsequent staining of timed samples with fluorescent antibody. By such techniques, "old" cell wall is distinguished from "new" and its fate can be followed visually for the first time. Our observations show that labelled antibody is not redistributed, once it is fixed to cell wall. New wall formation is initiated equatorially in each cell, such that half of each daughter cell wall is "old" and half "new". A previous theory of new cell wall formation in bacteria, by diffuse intercalation with old wall, has thus been shown to be untenable -- at least for streptococcus. Our findings are incorporated in a manuscript which has been submitted to Science.

(b) Parameters of the long chain reaction. Work on improvement and simplification of the long chain test for type-specific antibody to Group A streptococci, as described last year (1960; NIAID Serial No. 65-A, part B), culminated in a manuscript which has been accepted for publication. (See Part B. this project).

(c) Fluorescent antibody as a cell wall marker in following effects of antibiotics. Growth in fluorescein-labelled group- or type-specific homologous antibody is being used to visualize the effects of added antibiotics on synthesis of antigenic cell wall components of streptococci. Preliminary observations indicate that chloramphenicol and tetracycline inhibit formation of serologically active M protein, thus confirming the biochemical work of others on inhibition of protein synthesis in the presence of these antibiotics. Chloramphenicol, as expected, does not inhibit formation of the principal cell-wall constituent, the "C" polysaccharide; and the observable effects of the other antibiotics on the synthesis of the antigen are under active study.

(d) Effect of M protein on phagocytosis of streptococci. Studies have been continued on the previously reported enhancement of phagocytosis of Group A streptococci by mouse leukocytes in the presence of added homologous M protein. M-containing extracts of Types 1, 6, 12 and 18, as well as of the previously studied Type 23, enhance phagocytosis, within a range of from 9% to 70%. Acid-extracted and purified M protein gives similar results, but M protein prepared by the picrate method (See 1960 Annual Report, NIAID Serial No. 65-A, part A) does not. Further investigations, using human leukocyte systems, are in progress.

(e) Streptococcal bacteriophages. Beginning investigations of bacteriophages of Group A streptococci have resulted in development of suitable media for demonstration of plaques and for culture of lysogenic strains. Available strains of Types 1, 2, and 3 streptococci have been tested for lysogenicity and for their capacities as indicator strains. Phages lytic

for streptococci of Types 1, 2, and 12 have been isolated from lysogenic strains and immune sera against them are being prepared. Accurate methods for titrations of streptococcal phages are being developed.

(f) Mechanism of the long chain reaction. By the method of growth in fluorescein-labelled homologous antibody followed by antibody removal, the site of long chain fragmentation has been shown to be in areas of new cell wall formation and not at antibody-coated sites when type-specific antibody is used. In group-specific antibody, in which long chains are not formed, separation between cocci appears to occur as readily at antibody-coated sites as at uncoated new cell wall sites. Fluorescein-labelled univalent antibody fragments are as capable as bivalent antibody of combining with cell wall antigens (as seen under the ultraviolet microscope), but do not result in long chain formation. In other systems, univalent antibody has been shown to inhibit enzyme activity as readily as does complete antibody. These results suggest, therefore, that long chain formation in the presence of type-specific antibody is a form of agglutination dependent on complete antibody and may not be enzymatic as proposed by other investigators.

Significance to bio-medical research and the program of the Institute. Streptococci of Group A are uncommonly well characterized antigenically as well as morphologically, culturally, and biochemically. Several components of their cell walls or other surface layers are antigenic, distinct, readily isolated, and have definite biologic or immunologic effects. These streptococci grow readily, aerobically, in many media including serum and homologous antiserum. They are thus unsurpassed, but surprisingly neglected, prototype subjects for studies of basic biologic and chemical phenomena among bacteria in general and gram-positive cocci in particular. Using them, we have been able to demonstrate such possibly general phenomena as: long-chaining in homologous antiserum as a form of agglutination; the formation of new bacterial cell wall from a equatorial region of activity and not by diffuse intercalation; and direct visual confirmation of effects of different antibiotics on formation of different cell wall or surface components. The implications of studies of this nature are widespread -- not only to growth, morphogenesis, and general bacteriology, but also to applications which may be useful in understanding the pathogenesis of, resistance to, the immunology of, and the sequelae of, streptococcal disease.

Proposed Course of Project: We will continue basic investigations of streptococci as indicated above and as new areas for study develop from current findings. Experiments on the intracellular fate of phagocytosed streptococci, using various modifications of the fluorescein-labelling technique, are already in progress. Similar methods are expected to permit following the conversion of streptococci to protoplasts. Using the long chain method, modified as reported in a forthcoming publication, we are studying the type-specific antibody response in young children after isolations of Type 12 streptococci, thus rapidly applying basic findings to epidemiologic investigation. The relation of streptococcal phages to their sensitive streptococcal hosts is being studied, with particular emphasis on identification of cell wall receptors for phage and on the role, if any, of phage sensitivity or lysogenicity to virulence or toxin production.

Part B Included: Yes

Serial No. NIAID 65

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Bell, J. A., Huebner, R.J., Rosen, L., Rowe, W.P., Cole, R.M., Mastrota, F.M., Floyd, T.M., Chanock, R.M. & Shvedoff, R.A.: Illness and microbial experiences of nursery children at Junior Village. To be published, Am.J. Hyg., 74, Nov.1961.

Hahn, J.J. and Cole, R. M.: Time and concentration relationships in the long chain reaction of Group A streptococci in homologous antiserum; and an improved method for evaluation of test results. To be published, J. Bact., 83, Jan. 1962.

Honors and Awards relating to this project:

None

Serial No. NIAID 66 A-J
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Epidemiologic Studies of Illnesses and Microbial Experience of Junior Village Nursery Children

Principal Investigators: Dr. Joseph A. Bell, Dr. Albert Z. Kapikian

Other Investigators: Dr. Robert J. Huebner

Cooperating Units: Virus and Rickettsial Diseases Section, LID (Dr. Chanock, Dr. Johnson), Epidemiology Laboratory Unit (Dr. Rosen), Oncolytic and Oncogenic Virus Unit (Dr. Rowe), Virology Section, Perinatal Research Branch, NINDB (Dr. Sever)

Man Years (Calendar Year 1961)
Total: 127/12
Professional: 32/12
Other: 95/12

Project Description:

This is a long term, intensive study of the illness and microbial experiences of nursery children at Junior Village, a District of Columbia Welfare Institution. It began in July 1955 and provides research material for many projects of which 66 A to J are reported herewith together.

(A) Basic Objectives: The general objective is to maintain under observation a population group suitable for epidemiologic study of occurrence of infection and disease and host-parasite-disease-relationships as they occur naturally in this group and as they can be altered by chemo prophylaxis and new vaccines. One of the chief interim objectives is the development of epidemiologic, clinical and laboratory tools and methods for studying infectious diseases. A concerted effort is being made to find new microbiologic agents which cause disease, methods of identification of these agents, modes of spread and methods for prevention and treatment of acute illnesses, particularly respiratory illnesses. The study is designed to shed light on the nature and scope of studies which should be pursued in further search for methods of control of acute infectious diseases particularly, the large mass of acute respiratory diseases, including the common cold.

Part B included ☒ Yes ☐ No

Methods Employed and Patient Material:

The study children are located in Southwest Washington, D.C. The daily population is now close to 130 white and negro babies six to 50 months of age who are in residence in Eisenhower Cottage and in the Infirmary Building for domiciliary care. The population turnover was frequent with an average of 5 - 6 new admissions per week. Children with illnesses are studied either in their domicile or the infirmary at Junior Village, or the Clinical Center at N.I.H. or at the D.C. General Hospital, depending upon the severity of illness and study interest.

A full-time physician (D.C. Welfare Dept.), three nurses (NIH), and two nurses' assistants (NIH) maintain constant medical surveillance of the children, record rectal temperatures once every day, collect specimens for laboratory study and prepare clinical records on each child each day, regardless of whether or not ill. Both throat and anal specimens for virus study are collected on admission, and once weekly from every child, and otherwise when indicated. Blood specimens are collected on admission, on discharge, six weeks after admission, every three months during residence, and under special circumstances.

The temporal relations between specific infections and illnesses have been determined, and in order to confirm the etiologic roll of these agents extensive serologic testing must be carried out. Due to the limited amounts of serum available, this has been delayed pending development of microserologic techniques. The technique of such testing for hemagglutination inhibition antibody has been perfected and a detailed epidemiologic study of adenoviruses is under way.

Major Findings:

Each of 10 projects is summarized.

Project 66 A The general epidemiology of illness and infection in the Junior Village Nursery.

The first three years of study embraced 479 normal children 6 - 35 months of age who lived under somewhat crowded conditions at Junior Village. In an average week, 53 children were under observation and of these an average of four per week were new admissions. The mean duration of residence was approximately 17 weeks per child. This project describes the study arrangement, the general epidemiology of illness and infections and their temporal interrelationships, while other projects (B-F) detail experiences with specific infections. During the early part of the three-year study, the intensity of observations was increased but thereafter it remained fairly constant and illnesses were observed by a full time medical staff who took rectal temperatures twice daily on all children. Specimens for serologic study were collected from all children on admission and at routine intervals thereafter. Throat and anal

specimens for viral and bacterial study were collected from all children on admission, at onset of illness and routinely at least once a week. During the latter part of this study project when observations were most intense some 4,000 laboratory specimens per month were collected and tested for viruses and bacteria.

Aside from 107 cases of rubeola and 102 cases of varicella, departures from normal health were identified on the basis of fever 100.6°F and were classified as questionable fevers (A) and definite illnesses (B or C) depending upon the height and persistence of fever and occurrence of associated clinical manifestations. The mean weekly definite illness (BC) attack rate was 21.5 per cent. It varied considerably from week to week and varied slightly by season but was nearly uniform by age, sex and race. However, the rates for definite illnesses (B) i.e., definite fevers without associated clinical findings, increased during the early part of the study as intensity of observation increased whereas the rates for definite illness (C) remained relatively constant throughout the study. The attack rates for definite illnesses, both B and C, decreased with duration of residence in the nursery probably indicating increased immunity to infection.

By laboratory isolation a total of 1718 and 2307 child-infections were found with 55 virus and 54 bacterial serotypes, respectively. The infection rates were similar by sex, but differed slightly by age and race for different infections. Adenovirus and enteric bacterial infection rates were notably high in the very young. In general, each child averaged a new bacterial or virus infection every 2 - 4 weeks and many unrecognized infections undoubtedly occurred. At least 10 new virus serotypes have been discovered in these studies and others first found elsewhere have been classified as the result of these studies.

There was a significant association between acute febrile illness (BC) and infections with adenovirus types 1, 3 and 5, myxovirus influenza A2 and para-influenza virus types 1 and 3 (hemadsorption types 2 and 1, respectively), polio-virus type 2, Coxsackie B virus type 3, Shigella sonnei phase 1, and group A beta-hemolytic streptococci types 4 and 12 and one or more of types 1, 2, 5 and 23. These associations were found using the mass of undifferentiated, definite illness rather than clinical entities, using somewhat arbitrary end points, and without using the as yet incomplete antibody studies which eventually will help determine if the time when an agent was first isolated indicates the time of first infection. These associations were of such a nature as to indicate an etiologic relationship; however, lack of association between illness and other specific infections does not necessarily imply that these agents lacked pathogenicity or that they could not induce illness under other circumstances.

Project 66 B An outbreak of Respiratory Syncytial (RS) Virus Pneumonia

This occurred during the four week period April 24 to May 21, 1960 among 36 (40%) of 90 institutionalized children between eight and 50 months of age. Twenty (90%) of 25 children located in the Infirmary and 16 (25%) of 65 in the Cottage developed pneumonia. A four day incubation period was adequate to explain the time of sequential occurrence of pneumonia cases. Respiratory Syncytial virus was isolated from 24 children and was shown to be significantly associated with the onset of a febrile illness of 100.6°F or greater in the Infirmary by both vertical and horizontal analysis as used in project 66 A. A significant association between the onset of pneumonia and the presence of RS virus was found in both the Cottage and the Infirmary, suggesting an etiologic relationship between the RS virus and pneumonia in this study.

Serologic study of pre and post outbreak sera indicated that 91% of the study group became infected with RS virus. No relationship between pre outbreak neutralizing antibodies and occurrence of pneumonia could be shown in this study.

The average highest fever among the 36 patients with pneumonia was 103°F (rectal) with a mean duration of fever of 100.6°F or greater of four days. Coryza and cough were predominant symptoms during the illness. On auscultation fine or medium inspiratory crepitant rales were heard in circumscribed areas on the chest.

It appears that RS virus is the cause of a common and important respiratory disease of young children.

Project 66 C Attenuated measles virus vaccine and gamma globulin

These study products were administered to 16 children while 17 randomly selected controls received the gamma globulin alone in an attempt to determine (1) if the gamma globulin could modify or eliminate the vaccine reactions reported by others, (2) if the formation of measles antibody could be induced by the measles vaccine plus gamma globulin, and (3) if the vaccinees could be protected from developing the naturally occurring disease. Six of 8 vaccinees who developed antibody after vaccination and 3 of 9 controls had the onset of febrile episodes of 100.6° or greater from 4 - 14 days after inoculation ($P > .05$). The febrile episodes experienced by the vaccinees tended to have higher mean and median maximum temperatures and also tended to be of longer duration than those experienced by the controls. It is probable that the febrile episodes of the vaccinees were due both to vaccine reactions and intercurrent infections since the controls tended to develop fewer and milder febrile episodes than the vaccinees; as a result the exact nature of the vaccine reactions could not be determined. However, the vaccine reactions

in this study did appear to be somewhat milder than those reported in other studies which used vaccine alone, as none of the vaccinees developed either Koplik spots or rash.

Thirteen of 14 vaccinees with no prevaccine measles HI antibody developed such antibody after vaccination. During a measles outbreak, all of 12 controls and only 1 of 10 vaccinees developed the naturally occurring diseases indicating that the vaccination had rendered significant protection ($P < .01$). The one vaccinee who developed measles was the only child who had failed to develop post vaccine HI antibody.

The HI test appeared to be quite sensitive as a measure of measles infection as 30 of 32 children without pre-outbreak HI antibody and only 1 of 71 with such antibody developed the naturally occurring disease.

Project 66 D An outbreak of acute infectious lymphocytosis.

The outbreak occurred between August and September, 1960, when at least 22 cases of acute infectious lymphocytosis occurred. A white blood cell count of 20,000 per cu mm was set as the minimum value for establishing the diagnosis. The leukocyte counts actually varied from 20,000 to 94,000 per cu mm with a relative lymphocytosis of at least 80% in most of the cases. After the outbreak had begun, routine white blood cell counts were recorded in the entire population twice a week originally, and subsequently once each week, in an attempt to define an incubation period of the disease, and also to present an accurate estimate of the duration of the lymphocytosis. As a result, in 10 of the children a previously normal leukocyte count was recorded before the actual onset of the lymphocytosis. Analysis of the outbreak from an etiological, epidemiological and clinical viewpoint is currently in progress. From the etiological viewpoint, specimens (throat, anal, blood, bone marrow) have been inoculated into numerous tissue culture systems in an attempt to isolate viruses present during the outbreak. Specimens have been inoculated into other media for study of bacteria, fungi and parasites. Facilities of a local hospital and at the Clinical Center have permitted intensive clinical study of individual cases. Analyses of these records is currently in progress.

Project 66 E An outbreak of Croup Associated (CA) virus infection.

This occurred in April and May of 1960 among the nursery residents of Junior Village. During this period, 20 initial isolates of CA virus were recovered. Analysis from an epidemiological, etiological and clinical viewpoint is in progress.

Project 66 F Epidemiology of Adenovirus Types 1, 2, 3 and 5 infection and disease.

The admission sera of many children have now been tested for neutralizing antibodies against adenovirus types 1, 2, 3 and 5. This

reflects the infection experience which occurred in children who lived in the general population of the more congested areas of Washington, D.C. It confirms that infection with these types occurred very early in the lives of these children. Some 40% had been infected with types 3 and 5 and 60 to 80% had been infected with types 1 and 2 by the time they were three to five years of age. The antibody studies also gave a large measure of confirmation to the view that the previously demonstrated (Project 66 A) temporal association between the first isolation of adenovirus types 1, 3 and 5 (not 2) and acute febrile illnesses in these nursery children was an etiologic association. The excess illness attack rates at the time of first isolation of type 1, 3 and 5 were manifest only among children with no prior homologous experience and not among children who had been previously infected as indicated by the presence of neutralizing antibody or prior virus isolation.

Project 66 G The Epidemiology of Poliovirus Type 2 and 3.

Virus infection and illness. The general study (66A) demonstrated a temporal association between polio 2 (not 3) infection and acute febrile (nonparalytic) illness. Serological studies are needed to determine relation of antibodies to infection and diseases.

Project 66 H A Study of a 12-Strain Respiratory Vaccine.

Clinically this vaccine gave protection against Asian influenza and nothing else. Serologic studies are needed to determine what other virus infections occurred which were homologous to the virus in the vaccine and to determine if the vaccine induced antibodies to the virus types in the vaccine.

Project 66 I Penicillin as a Chemoprophylactic Agent.

Daily doses of 300,000 units of penicillin were given orally to a randomly selected 20% of the children and clinically this did not appreciably influence occurrence of acute febrile illness and it did not influence occurrence of virus infection but did influence occurrence of streptococcal infection. Further study of data is needed.

Project 66 J A Comparative Study of Reactions to DPT.

Two DBS accepted products which gave extremely different toxic reactions in mice were given to alternate children and to date no difference in reactions has been noted following 95 injections.

Significance to Bio-medical Research and the Program of the Institute:

Although the study is deliberately limited to a group of young children who have a high proportion of susceptibles to various microbial infections, it shows some of the potentialities of such infections in

under acceptable practices. This project represents a systematic approach to determining the various etiologies of acute febrile illnesses so as to guide research on development of methods for control.

The project provides a source of specimens for finding new causes of illness, for studying their etiologic significance and for testing methods of control. It is significant that long term Junior Village studies of host-parasite-disease-relationships are being carried out on the base of illness data obtained from military and absenteeism from schools and industry. The studies are of such a nature that they are not likely to be conducted by other than a government group. The studies are progressing meticulously and fairly satisfactorily to the investigators.

It is obvious that the many acute febrile respiratory diseases and fevers of undetermined origin which are a major public health problem are caused by a great multiplicity of etiologic agents. To prevent or ameliorate these illnesses by vaccine prophylaxis requires a multi-polyvalent vaccine to be developed. Each of these known agents is producing a very large proportion of these diseases. It seems timely to initiate controlled epidemiologic studies in the use of multivalent vaccines and their possible enhancement or interference with each other.

Proposed Course of Project:

Clinical epidemiological observations are being continued together with weekly collection of throat and stool specimens for virus study and collection of routine blood samples. Blood samples will be tested extensively for specific antibodies and when infections or illnesses of interest are found, virus isolation studies will be carried out to pin point the infection and its relation to illness. It is planned to continue the study for as long as it appears profitable.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B

Honors, Awards and Publications

Rosen, Leon, Bell, Joseph A., and Baron, Samuel

Four Newly Recognized Adenoviruses

Proc. Soc. 107:434-437, 1961

Kapikian, A.Z., Bell, J.A., Mastrota, F.M., Johnson, K.M., Huebner, R.J., and Chanock, R.M.

An Outbreak of Febrile Illness and Pneumonia Associated with Respiratory Syncytial Virus Infection

Amer. Jour. of Hygiene, Nov. 1961

Knight, Vernon, Kapikian, A.Z., Kravetz, H.M, Chanock, R.M., Morris, J.A., Huebner, R.J., Smadel, J.E., and Evans, H.E.

Ecology of a Newly Recognized Common Respiratory Agent RS Virus (Combined Clinical Staff Conference at the National Institutes of Health)

Annals of Internal Medicine, Vol. 55, No. 3, Sept. 1961

Kapikian, A.Z.

Editorial: Influenza Vaccination

J.A.M.A. Nov. 11, 1961

Bell, J.A., Huebner, R.J., Rosen, L., Rowe, W.P., Cole, R.M., Mastrota, F.M., Floyd, T.M., Chanock, R.M., and Shvedoff, R.A.

Illness and Microbial Experiences of Nursery Children at Junior Village

Amer. Jour. of Hygiene, Nov. 1961

Part B (Cont.) Honors, Awards and Publications

Kapikian, A.Z., Bell, J.A., Rosen, L., and James, R.C. (Not NIH)

Attenuated Measles-Virus Vaccine: Clinical, Antigenic and Prophylactic Effects of the Vaccine when Administered with Gamma Globulin

J.A.M.A. (To be published)

Honors and Awards related to this project:

Arranged and conducted symposium involving presentation of seven papers on Acute Respiratory Diseases at Epidemiology Section Meeting of A.P.H.A.

Served on Governing Council, A.P.H.A.

Served on Medical Advisory Council, D. C. Welfare Department

Lecture - National Academy of Sciences - April, 1961
"A Search for Microbial Causes of Common Illnesses"

Appointed Member of Committee on International Quarantine for WHO

Member Armed Forces Epidemiological Board Influenza Commission

Invitational Lecture Influenza Commission, British Medical Research Council, London, England

Committee on Control and Prevention of Infections in the Clinical Center, N.I.H.

Serial No. NIAID 66 K
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Epidemiological Studies on Efficacy and Safety of Influenza Vaccine in General Population Groups

Principal Investigators: Joseph A. Bell, Robert N. Philip and Dorland J. Davis

Other Investigators: M.O. Beem, P.M. Beigelman, J.I. Engler, G.W. Mellin, J.H. Johnson, A.M. Lerner

Cooperating Units: None

Man Years (Calendar year 1961)
Total: 1+/12
Professional: < 1/12
Other: 1/12

Project Description:

This was a 1951-56 controlled study of aqueous and mineral oil adjuvant monovalent influenza vaccines, Type A, A1 and B, among some 19,000 people in general population groups of 26 local residential areas of Norfolk and Arlington, Virginia, and Montgomery County, Maryland. An outbreak of Influenza B was observed in 1952, Influenza A in 1953, and Influenza B in 1955. A preliminary report on protection in the 1952 and 1953 outbreaks was published in 1954 and comprehensive reports on the five year study were published in 1961 embracing (1) the general epidemiology of influenza and acute febrile respiratory illnesses, (2) laboratory observations, and (3) vaccine reactions. These were noted in last year's annual project report. A comprehensive report on the amount and duration of vaccine protection remains to be completed.

Major Findings:

Influenza constitutes such a small proportion (8-15%) of the clinically undifferentiable acute febrile illnesses of two or more days duration, that for the total five year study period the effects of a highly effective influenza vaccine would hardly be discernible. It is obvious that the aqueous vaccines (300 CCA units) gave no detectable protection but the oil adjuvant vaccines (100 CCA units) gave definite protection during the three outbreaks, and this protection apparently lasted 2 - 3 years. On the other hand the amount of protection in the 1955 outbreak was apparently so small as to be of no consequence.

Part B included ☐ Yes ☒ No

Significance to Bio-medical Research and the Program of the Institute:

There is no evidence that commercially available influenza vaccines (aqueous) will protect for longer than one year. It is impractical and not worth the effort to get everybody vaccinated every year with available products. What is needed is a vaccine that will protect for many years. These studies have demonstrated that for this end the mineral oil adjuvant vaccines are the most promising products available to date.

Proposed Course of Project:

Aside from a possible follow up of latent reactions, the field study is completed. Analysis of data will continue (1) in an effort to develop epidemiologic methods for better evaluation of amount and duration of protection and (2) to study the epidemiology of acute respiratory diseases in general population groups.

Serial No. NIAID 66 L
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Epidemiological Studies on Efficacy and Safety of Asian Influenza Vaccine in General Population Groups 1957-1958

Principal Investigator: Raymond Seltser (Johns Hopkins University)

Other Investigators: Philip Sartwell (JHU) and Joseph A. Bell

Cooperating Units: Johns Hopkins University, Baltimore, Maryland and National Institutes of Health

Man Years (Calendar Year 1961)
Professional: <1/12
Other: None

Project Description:

Background and Objectives: In the Fall of 1956, no new vaccine groups were added to project 66 K as it was prophesied that the type A, A1, and B strains used in the vaccine would not again become epidemic for many years. In July 1957, a new Asian strain A2 virus promised to become epidemic in the United States. We carried out an Asian strain vaccine trial in volunteers later challenged with live Asian influenza virus and demonstrated a 65% protective effect. We provided an Asian strain vaccine and direct assistance to Johns Hopkins University to carry out a placebo controlled study in part of our Montgomery County study group for the purpose of determining (1) the effectiveness of the vaccine in preventing influenza (2) the frequency, nature and severity of reactions and (3) to study the general epidemiology of influenza.

Methods Employed -- Patient Material: Same as for project 66 K with the exception that only 3180 persons were given either the monovalent aqueous Asian influenza vaccine or the placebo control.

Major Findings: This is the first good study in general population groups of influenza vaccine reactions wherein a placebo control was used thus enabling a reasonably good approximation of the nature and frequency of vaccine induced reactions. Vaccine induced systemic reactions occurred in 8.5% of vaccinees but the frequency of incapacitating reactions was only 4.3%. Fortunately, progressively smaller

Part B included /X/ Yes / / No

doses were used in children <12 years of age, otherwise the frequency of reactions would have been much higher. Unfortunately, a polyvalent product was not used, hence one can extrapolate only to the extent that this is probably a minimum reaction rate. It is important that the febrile systemic reactions that could be attributed to the vaccine all had onset within 24 hours after vaccination. The amount of protection was estimated to be 65% and the vaccine protected all age groups except young children < 3 years old. In this study again it was evident that the primary spread of influenza in the community was predominantly in the Junior and Senior High schools. From here it spread to other members of their households.

Significance to Bio-Medical Research and the Program of the Institute:

Aside from the excellent data on vaccine reactions obtained in this placebo controlled study it is comforting to have a conclusive demonstration that when a new pandemic strain of influenza virus was discovered in early summer, a vaccine of demonstrated effectiveness in general population groups was prepared and effectively administered before the Fall outbreak. It is interesting that a 65% protection from a single dose was observed in this study and a previous study showed that a single dose of a similar Asian vaccine gave 65% protection to artificially challenged volunteers.

Proposed Course of Project:

Concluded

Part B Included: Yes

Serial No. NIAID 66 L

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B

Seltser, Raymond (JHU), Sartwell, Philip (JHU), Bell, J.A.

A Controlled Test of Asian Influenza Vaccine in a Population of Families

American Jour. of Hyg. (To be published)

Serial No. NIAID 66 M
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Hemagglutination Inhibition (HI) Antibody to Type A and B Influenza 8 - 9 years after Vaccination with 100 CCA Units of Monovalent Mineral Oil Adjuvant Vaccine

Principal Investigator: Fred M. Davenport, Albert V. Hennessy and Joseph A. Bell

Other Investigators: R.N. Philip, R.A. Shvedoff

Cooperating Units: Cooperative Study, N.I.H. and University of Michigan

Man Years (Calendar year 1961):
Total: <1/12
Professional: < 1/12
Other: < 1/12

Project Description:

In 1951 and 1952, several hundred N.I.H. employees were by a strict random sampling process, separated into groups each of which was given 1 dose of a mineral oil adjuvant influenza vaccine either Type A or B. All were bled pre and post vaccination and all who could be found have been bled several times since. These people were studied for local and general reactions and serologically for HI antibody responses.

In the fall of 1960, 110 vaccinees were found and re-bled and the pre and all post vaccine sera from these persons were titered for HI antibody to all antigens used in the vaccines.

Major Findings:

Both local and general reactions from the vaccine were strikingly mild and infrequent and immediate antibody responses were excellent. The set-up of the study was such that in spite of any interim infection or vaccination, there can be no reasonable doubt but that the small dose of oil adjuvant vaccine given in 1951 or 1952 influenced the antibody pattern in 1960, 8 or 9 years after vaccination.

Part B included ☒ Yes ☐ No

Significance to Bio-medical Research and the Program of the Institute:

These findings and those of study project 66 K constitute the chief and practically only basis for continued studies with oil adjuvant influenza vaccines. Although there are some detriments, the oil adjuvant product is by far the most promising influenza prophylactic available.

Proposed Course of Project:

Concluded

Part B Included: Yes

Serial No. NIAID 66 M

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B

Davenport, Fred (Not NIH), Hennessy, Albert (Not NIH) and
Bell, Joseph A.

Immunologic Advantages of Emulsified Influenza Virus Vaccine

Military Medicine (to be published)

Serial No. NIAID 66 N
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Inoculation of Parainfluenza Virus Type 3 in Adult Volunteers

Principal Investigator: Albert Z. Kapikian

Other Investigators: Robert M. Chanock, Joseph A. Bell, Robert J. Huebner, Thomas E. Reichelderfer (formerly at NIH)

Cooperating Units: Dr. Thomas G. Ward (Director, Virus Research, Microbiological Associates, Bethesda, Maryland), Dr. Roger M. Cole (Chief, Bacteriological Unit, LID)

Man Years (Calendar 1961)
Total: 1/12 Professional

Project Description:

Objectives: To determine the role of parainfluenza virus type 3 in adult respiratory disease.

Methods Employed - Patient Material:

Volunteers were healthy male inmates at the Patuxent Institution of The Maryland State Board of Correction at Jessup, Maryland.

Major Findings:

In an attempt to see whether parainfluenza virus type 3 would produce illness in adults, 28 adult volunteer subjects were inoculated with either the virus or a sterile salt solution. Twelve of the 17 volunteers inoculated with the virus, and none of the 11 who received the placebo inoculum, developed laboratory evidence of infection with parainfluenza 3 virus. Nine of the virus-inoculated volunteers and only 1 of the 11 in the control group developed an acute respiratory illness. However, 3 of the 9 virus-inoculated subjects who became ill failed to demonstrate laboratory evidence of infection with parainfluenza 3. The afebrile, "cold-like" illness observed in the 6 infected volunteers was characterized by mucoserous nasal discharge, nasal obstruction, sneezing, dry cough, and erythematous nasal and pharyngeal mucous membranes. The mean incubation period was 2.2 days.

Part B included ☒ Yes ☐ No

Significance to Bio-Medical Research and the Program of the Institute:

This was the first volunteer study in the United States with parainfluenza virus type 3. It has furnished evidence consistent with the view that parainfluenza virus type 3 can produce illness in human adults. One other study in volunteers with parainfluenza type 3 has been carried out in England.

Proposed Course of Project:

This study with parainfluenza virus type 3 at the Patuxent Institution has been concluded.

Part B Included: Yes

Serial No. NIAID 66 N

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Kapikian, A.Z., Chanock, R.M., Reichelderfer, T.E. (formerly at NIH) Ward, T.G. (formerly Professor of Virology, Notre Dame University, now Director of Virus Research, Microbiological Associates, Bethesda, Maryland) Huebner, R.J., and Bell, J.A.

Inoculation of Human Volunteers with Parainfluenza Virus Type 3

J.A.M.A. Vol. 178, No. 6, Nov. 1961

Serial No. NIAID 66 0
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: The Etiology of Respiratory Disease on a College Campus

Principal Investigator: Dr. Albert Z. Kapikian

Other Investigators: Dr. Karl Johnson, Dr. Robert Chanock, Dr. Joseph Bell, Dr. Robert Huebner

Cooperating Units: University of Maryland Infirmary

Man Years (Calendar Year 1961)
Total: <1/12
Professional: <1/12
Other: <1/12

Project Description:

Objectives: (1) To determine the role of known viruses as etiologic agents in a young adult population; (2) To find new agents which might be associated etiologically with respiratory disease.

Patient Material:

With the cooperation of the University of Maryland Infirmary, a study was undertaken in October 1958 to determine the role of viruses in the etiology of respiratory diseases in a young adult population. Patients who appeared with complaints referable to their respiratory tract were examined. Throat swabs and acute and convalescent bloods were also obtained. An attempt to have a control group of nonrespiratory patients was unsuccessful. The throat specimens were tested for viruses in appropriate tissue culture and the blood was tested for CF antibody against a battery of respiratory virus antigens.

Major Findings:

The finding of a new parainfluenza virus type 4 was presented previously.

Two hundred ten patients with respiratory disease were seen — among these, 49 (23%) had fever of 99°F or greater. Virus isolation attempts were markedly unsuccessful, as only 2 virus isolations were

Part B included ☐ Yes ☒ No

made. However, paired bloods were obtained from 122 of the 210 students. Thirty or 25% of the 122 students showed a four-fold rise to one of the following respiratory viral antigens by CF test: Parainfluenza 1, 3, 4 ; Adenovirus; Influenza A and B; Respiratory Syncytial Virus; Herpes Simplex. Of the 30 showing CF antibody rises, over one-half (53%) were to Influenza B, 17% to Respiratory Syncytial, 10% to Parainfluenza 1, and 3% to each of the other antigens. The illnesses of the patients with the rises to each of the antigens was not associated with any specific clinical syndrome.

In addition, the paired sera were also tested for CF antibody rises against a battery of enterovirus antigens (ECHO 9, 20, Cosxackie A2, 4, 6, 21, B3, Pett). No rises were found. However, it was of interest that only 1 - 15% had CF antibody (1:8 or >) to the ECHO 9, 20, Coxsackie A2, 4, 6, and B 3 viruses while 35% and 68% had CF antibody (1:16 or >) to the Coxsackie A 21 and Pett viruses, respectively. These findings may indicate one or a combination of the following possibilities in this population: (1) Previous infection by the first group of agents was rare whereas such infection by Coxsackie A 21, Pett or closely related viruses was common; (2) Complement fixing antibody titers diminish below detectable levels within a few years following infection; thus the results indicate only that infection by the second group of agents was more recent than by the former; (3) In comparison to the antigens of the first group, the Coxsackie A 21 and Pett antigens were more broadly reactive as indicators of CF antibody following enterovirus infection regardless of serotype.

Significance to Bio-medical Research and the Program of the Institute:

This project attempted to show the etiology of respiratory diseases with respect to viruses. It is seen clearly that serologically, only 25% of the respiratory diseases could be associated with viruses and if this had been a year when influenza B was not prevalent, the figure might have been even lower. The task of further attempting to find those agents which are causing viral respiratory diseases is apparent. In addition, it is obvious that further studies are needed to determine the value of the CF test as a method for measuring infection with the newer enteroviruses.

Proposed Course of Project:

There are no plans to renew this study at the present time. However, additional serologic studies may be carried out if indicated.

Serial No. NIAID 66 P
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: The Role of Viruses in the Etiology of Ear Disease

Principal Investigator: Dr. Albert Z. Kapikian

Other Investigators: Dr. Joseph A. Bell, Dr. Robert J. Huebner

Cooperating Units: Washington Hospital Center, Ear, Nose and Throat Department

Man Year (Calendar Year 1961)
Total: 0/12
Professional:
Other:

Project Description:

For many years, it has been stated that viruses were responsible for many nonbacterial ear infections such as serous otitis media. It was attempted to study patients with serous otitis media to determine the role of viruses in this disease.

Patient Material:

Patients at the Washington Hospital Center ENT clinic with serous otitis media were examined. Fluids from the middle ear were tested in appropriate tissue culture lines. Acute and convalescent bloods were obtained.

Major Findings:

Thus far only a limited number of specimens from the ear have been tested with no virus yet isolated. Serologic tests on the sera are in progress.

Significance to Bio-medical Research and the Program of the Institute:

The role of viruses in ear disease is uncertain. This project attempts to clarify their role in the etiology of middle ear disease, especially serous otitis media.

Proposed Course of Project:

It is planned to renew attempts to obtain appropriate specimens during the year.

Part B included ☐ Yes ☒ No

Serial No. NIAID 67
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Studies on enteroviruses, adenoviruses, reoviruses

Principal Investigator: Dr. Leon Rosen

Other Investigators: Dr. Joseph A. Bell, Dr. Frank L. Black, Mrs. Janet Hovis, Dr. Robert J. Huebner, Dr. Karl M. Johnson, Mr. Jerome Kern, Dr. D. Mendez-Cashion, Dr. Albert B. Sabin, and Dr. David A. J. Tyrrell

Cooperating Units: D. C. Welfare Department; Children's Hospital Research Foundation, Cincinnati, Ohio; School of Medicine, University of Puerto Rico, San Juan; Common Cold Research Unit, Salisbury, England; Yale University School of Medicine, New Haven, Connecticut; Laboratory of Clinical Investigation, NIAID

Man Years: (Calendar Year 1961)

Total:	88/12
Professional:	34/12
Other:	54/12

Project Description:

Objectives: To determine the role in human biology of enteroviruses, adenoviruses, and reoviruses. To elucidate the natural history of these viruses.

Methods Employed: Collaborative studies with institutions outside of the NIH and with various intramural laboratories are undertaken as opportunities are presented.

During 1961 studies were continued on the Junior Village nursery group which has been described in detail previously. The studies with the Department of Pediatrics at the University of Puerto Rico and

Part B Included Yes

Part A Methods Employed (continued)

also those with Children's Hospital Research Foundation of Cincinnati were continued. A study on measles was undertaken in collaboration with the Yale University School of Medicine, New Haven; a human volunteer study with ECHO virus type 20 was carried out in collaboration with the Common Cold Research Unit, Salisbury; and a human volunteer study on reoviruses was done in collaboration with the Laboratory of Clinical Investigation.

Major Findings: Intensive study of hemagglutination by enteroviruses was continued during the year. Hemagglutinins are now known for 22 enterovirus serotypes - one-half of these were first found as a result of investigations in this unit.

Two new enterovirus serotypes were recognized during the year and these are currently in the process of being worked up for submission to the Enterovirus committee. In addition, five new enterovirus serotypes isolated from persons with respiratory disease by Dr. K. Johnson are being worked up in collaboration with him.

The relationship of "Pett" virus to Coxsackie A type 24 was recognized during the year. Also, a hemagglutinating enterovirus recovered from an outbreak among military recruits by Dr. K. Johnson was identified in this unit as "Coe" virus (Coxsackie A type 21).

Human volunteer studies carried out in collaboration with Dr. D. A. J. Tyrrell and other members of the Common Cold Research Unit in England showed that ECHO type 20 virus (JV-1) was capable of inducing illness in such volunteers.

Studies carried out in collaboration with the Department of Pediatrics of the School of Medicine, University of Puerto Rico, provided presumptive evidence that acute cerebellitis in children can be caused by poliovirus type 1.

Several new adenovirus serotypes (in addition to the four recently described) were recognized during the year and are currently being worked up.

Virus isolation and serological studies of the "newer" adenovirus serotypes have shown that these viruses are very common in the Junior Village population. In contrast to the "lower" adenovirus serotypes, the "newer" types are found almost exclusively in the feces.

Human volunteer studies with each of the three reovirus serotypes were carried out in collaboration with personnel of the Laboratory of Clinical Investigation. Although very little illness was observed in these experiments much valuable data on the biology of reoviruses in humans was obtained.

Serial No. NIAID 67-

Part A Major Findings (continued)

The measles HI test developed in this unit in 1960 was used to test sera collected under unique epidemiologic conditions on the island of Tahiti. These studies, carried out in collaboration with Dr. F. Black, showed conclusively that the stable high-titered antibodies commonly observed in this disease were not the result of repeated exposure to the virus. Also, it was shown that canine distemper antibodies in human sera are the result of measles infection and are not the result of exposure to canine distemper virus.

Significance to the Program of the Institute: The role of the numerous enteroviruses, adenoviruses, and reoviruses in human biology is only beginning to be studied. Data obtained thus far indicate that a number of these viruses are quite important in human pathology. Further progress in this field depends to a great extent on the development of simple laboratory procedures to cope with the large number of viruses now known to belong to these groups.

Proposed Course of the Project: The study of these three families of viruses will be continued both in the field, as opportunities are presented, and in the laboratory. Laboratory studies will be devoted primarily to the orderly classification of existing and newly recognized serotypes and to the development of simple in-vitro techniques.

Part B Included: Yes

Serial No. NIAID 67

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Rosen, L.: Hemagglutination and Hemagglutination-Inhibition with Measles Virus. Virology, 13:139-141, 1961
2. Buckland, F. E., Bynoe, M. L., Rosen, L., and Tyrrell, D. A. J.: Inoculation of Human Volunteers with ECHO Virus Type 20. Brit. Med. Jour., 1:397-400, 1961
3. Johnson, K. M., Bloom, H. H., Rosen, L., Mufson, M. A., and Chanock, R. M.: Hemagglutination by Coe Virus. Virology, 13: 373-374, 1961
4. Rosen, L. and Kern, J.: Hemagglutination and Hemagglutination-Inhibition with Coxsackie B Viruses. Proc. Soc. Exp. Biol. and Med., 107:626-628, 1961
5. Rosen, L., Baron, S., and Bell, J. A.: Four Newly Recognized Adenoviruses. Proc. Soc. Exp. Biol. and Med., 107:434-437, 1961
6. Black, F. L. and Rosen, L.: Patterns of Measles Antibodies in Residents of Tahiti and their Stability in the Absence of Re-exposure. Jour. Immunol. In Press.
7. Kapikian, A. Z., Bell, J. A., Rosen, L., and James, R. G.: Attenuated Measles-Virus Vaccine: Clinical, Antigenic and Prophylactic Effects of the Vaccine when Administered with Gamma Globulin. Jour. Amer. Med. Assn. In Press.
8. Rosen, L.: Measles on Tahiti. Amer. Jour. Dis. Child. In Press.
9. Bell, J. A., Huebner, R. J., Rosen, L., Rowe, W. P., Cole, R. M., Mastrota, F. M., Floyd, T. M., Chanock, R. M., and Shvedoff, R. A.: Illness and Microbial Experiences of Nursery Children at Junior Village. Amer. Jour. Hyg. In Press.
10. Rosen, L.: Hemagglutination-Inhibition Antibody Responses in Human Adenovirus Infections. Proc. Soc. Exp. Biol. and Med. In Press.

Serial No. NIAID 67

Part B Honors, Awards, and Publications

Honors and Awards relating to this project:

1. Invited speaker, International Conference on Measles Immunization, Washington, D. C., November, 1961

2. Invited to be a member of a World Health Organization committee on measles immunization, November, 1961

Serial No. NIAID 67A
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Studies on Viruses of the Enteric Tract of Cattle

Principal Investigator(s): Dr. F. R. Abinanti and Dr. Leon Rosen

Other Investigators: None

Cooperating Units: Dairy Husbandry Department, University of Maryland; Home of Correction, Jessup, Maryland

Man Years (calendar year 1961):
Total: 6/12
Professional: 3/12
Other: 3/12

Project Description:

Objectives: To investigate the biologic properties and natural history of the viruses inhabiting the enteric tract of cattle.

Methods Employed: A longitudinal study of dairy cattle on three different farms has been carried out for approximately two years. Monthly fecal swabs and serum specimens are obtained from each animal and these are studied in the laboratory by a variety of virus isolation and serologic procedures.

Major Findings: Studies of the three reovirus types and hemadsorbing (HADEN I) virus have been extended to learn more of the natural history of these agents. Work is progressing to serologically distinguish other viruses from this group of cattle. No definitive results are availabe at this time.

Significance to the Program of the Institute: The recovery of viruses (such as reoviruses) from cattle which are antigenically identical with those which occur in man is of obvious interest. Aside from the possible importance of cattle as potential sources of infection, a number of aspects of the natural history of these agents can be clarified by their study in lower animals. In addition, viruses recovered from cattle which are analogous, but not identical, to those of humans also proved a mechanism for gaining useful information about the latter agents.

Part B included: Yes

Proposed Course of the Project: When definitive serologic differences have been established for a group of these viruses, they will be studied for their properties to determine to which virus group they belong. The relationship, if any, to human viruses in comparable virus groups will be investigated.

Part B Included: Yes

Serial No. NIAID 67 A

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Abinanti, F. R., Respiratory and Enteric Viruses in Man and Animals. Public Health Reports 76: (10) October 1961.

Rosen, L., and Abinanti, F. R., Recovery of a Hemadsorbing Virus (HADEN) from the Gastrointestinal Tract of Calves. Virology 14:(2), June 1961.

Serial No. NIAID 67-B
1. Infectious Diseases
2. Epidemiology
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Studies on eosinophilic meningitis in the Pacific Area

Principal Investigator: Dr. Leon Rosen

Other Investigators: Dr. Raid Chappell, Dr. Gert L. Laqueur, Dr. Betty-June Myers, Dr. Elvio Sadun, Dr. Gordon Wallace, and Dr. Paul P. Weinstein

Cooperating Units: Institut de Recherches Medicales de la Polynesie Francaise, Papeete, Tahiti; St. Francis Hospital, Honolulu, Hawaii; Department of Health, State of Hawaii; Communicable Disease Center, USPHS; Laboratory of Parasitic Diseases, NIAID; Laboratory of Experimental Pathology, NIAMD; McGill University, Montreal, Canada; Department of Medical Zoology, Walter Reed Army Institute of Research

Man Years: (Calendar year 1961)

Total:	4/12
Professional:	2/12
Other:	2/12

Project Description:

Objectives: To determine the etiology and method of transmission of eosinophilic meningitis in the Pacific Area.

Methods Employed: Field investigations were carried out in both French Polynesia and in Hawaii during the year. Also, laboratory studies were undertaken in Bethesda with several helminthic parasites which were under suspicion for epidemiologic reasons.

Major Findings: At least several hundred cases of eosinophilic meningitis occurred on the islands of Tahiti and Raiatea in French Polynesia during 1961. In the course of the studies on Tahiti an attempt was made to determine if the disease also

Part B Included Yes

occurred in the Hawaiian Islands. As a result of this investigation it was learned that two patients who had died in a mental hospital about one year previously had had an eosinophilic meningoencephalitis. The brain of one of these patients had been preserved and in this specimen a number of young adult nematodes were found in both the brain substance itself and in the meninges. These parasites, which included an intact male, were identified as *Angiostrongylus cantonensis* (Chen) Dougherty, a metastrongylid lung-worm of rats. The presence of material foreign to the central nervous system in the brain of the second patient and a cellular reaction similar to that found in the first case suggested that this individual also was infected with the same parasite.

Cerebrospinal fluid obtained prior to the death of the first patient showed changes typical of those seen with eosinophilic meningitis on Tahiti. However, the mental condition of both patients prior to their death and their other concurrent pathology were such that it was not possible to determine if they had had symptoms similar to those of the Tahitian patients or if their death had been due to the meningoencephalitis. It was also not possible to determine how either patient might have become infected with the helminth.

Various species of rats are the only known natural hosts of the adult form of *A. cantonensis* and terrestrial slugs and snails are the only known intermediate hosts. The extensive epidemiologic data from the outbreak on Tahiti are not consistent with the life cycle of *A. cantonensis* as it is presently known. Thus, the population there affected by the disease does not intentionally eat terrestrial molluscs. Moreover, so little raw green vegetation is eaten by the majority of individuals that it is difficult to conceive of such molluscs being ingested accidentally by a significant number of persons. It is possible, of course, that the Tahitian patients could have been infected with *A. cantonensis* by means of a presently unknown intermediate or transport host.

Preliminary attempts to determine the relationship of *A. cantonensis* to the disease on Tahiti by serologic means have proven disappointing. Thus, convalescent sera from Tahitian cases almost all gave a positive reaction in a complement-fixation test with an antigen prepared from *A. cantonensis* while sera from recent arrivals on the island did not. However, almost all sera from long-time residents of the island who had no history of having had eosinophilic meningitis also gave a positive reaction. Moreover, exactly parallel results with the three groups of sera were obtained with an antigen prepared from a larval nematode found in fish.

Part A *Major Findings* (continued)

Laboratory experiments were undertaken with two helminths recovered from the flesh of fish in Tahiti to determine if these parasites could produce a meningitis in experimental animals. These experiments gave negative results although it was found that one of the organisms (*Anisakis* sp.) could penetrate the gut wall of guinea pigs.

Rats naturally infected with *A. cantonensis* were captured alive in Hawaii and shipped to Bethesda in order to provide source material for a laboratory strain of this parasite. The parasite developed successfully in local slugs (*Limax maximus* and *Deroceros reticulatum*) and gave rise to normal infections in laboratory rats.

Significance to Program of the Institute: Large numbers of cases of eosinophilic meningitis continue to occur in the Pacific Area and the disease has now been recognized in Hawaii. It is obviously of great interest to determine the etiology and source of this disease so that appropriate preventive measures can be undertaken.

Proposed Course of Project: Epidemiologic field studies will be continued during 1962 with the hope of obtaining a definitive lead as to the source of eosinophilic meningitis in French Polynesia. In addition, experimental and serologic studies with *A. cantonensis* will be continued in order to try to determine what proportion of the cases of eosinophilic meningitis in the Pacific Area is caused by this organism.

[illegible]

Serial No. NIAID 67-B

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Rosen, L., Laigret, J., and Bories, S.: Observations on an Outbreak of Eosinophilic Meningitis on Tahiti, French Polynesia. Amer. Jour. Hyg., 74:26-42, 1961

2. Rosen, L., Chappell, R., Laqueur, G. L., Wallace, G. D., and Weinstein, P. P.: Eosinophilic Meningoencephalitis of Man Caused by a Metastrongylid Lung-worm of Rats. Jour. Amer. Med. Assn. In Press.

Honors and Awards relating to this project:

1. Invited to present a paper entitled "Observations on an Outbreak of Eosinophilic Meningitis on Tahiti, French Polynesia" at the Tenth Pacific Science Congress, Honolulu, Hawaii, August, 1961

2. Invited to participate in a special symposium on geographic neurology organized by the World Federation of Neurology, Rome, Italy, September, 1961

3. Invited dinner speaker at the annual meeting of the Helminthological Society of Washington; subject: "Eosinophilic Meningitis in the Pacific Area," October, 1961

4. Letter (March 31, 1961) from the Governor of French Polynesia to the Surgeon General, USPHS, expressing appreciation for the studies on eosinophilic meningitis carried out on Tahiti

Serial No. NIAID 67 C
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: A Study of Squamous Cell Carcinoma (Cancer Eye) in Cattle.

Principal Investigators: Dr. F. R. Abinanti, Dr. W. Gay (DRS)
Dr. M. Stanton (NCI)

Other Investigators: Dr. R. J. Huebner

Cooperating Units: Bay Manor Farms (owner Mr. Otis Smith), Lewes, Delaware; M.D. Anderson Hospital (Dr. Russell), Houston, Texas

Man Years: (Calendar year 1961)
Total: 9/12
Professional: 6/12
Other: 3/12

Project Description:

Objectives: There are approximately 600 Hereford cattle on the Bay Manor Farms, Lewes, Delaware, and about 30 per cent of these cattle have precancerous or cancerous lesions of the eyes and orbital appendages. The principal efforts are to determine if a virus is responsible for this condition and to study the natural history of the disease.

Methods Employed: Monthly inspections are made of the herd and a photographic record is being made of the progress of the lesions. At this time eye swabs are taken and blood for possible future serological studies. Dr. William Gay surgically removes affected tissues at intervals which are examined histologically by Dr. M. Stanton, and virologically by Dr. F. R. Abinanti. Further frozen sections will be examined by the fluorescent technique to explore the possibility of there being a specific antigenic material present in the cancers.

A sero-epizootiological study of cattle with and without "cancer eye" has been planned in cooperation with the M.D. Anderson Hospital "Cancer Eye" research group under Dr. William Russell. Several hundred sera will be collected by this group from cattle with cancer eye and matched controls. The specimens will be tested for antibodies vs. bovine viruses and for some of the mouse, chicken and rabbit tumor viruses.

Part B included: No

Major Findings: Six virus recoveries have been made from the lacramel secretions of these cattle. Serological studies will be conducted with these viruses to determine their possible role in cancer eye etiology. Pathogenesis studies will be done later if these agents appear to play a role in the disease. All other avenues as outlined in last year's report, have proved fruitless.

Significance to the Program of the Institute: Provides an opportunity to explore the possible viral etiology of squamous cell cancinoma. The Bay Manor herd provides excellent nearby material for epidemiological studies of the natural history of the cancer itself.

Proposed Course of the Study: Collection of field specimens has been discontinued in view of the essentially negative results following extensive testing of specimens in the laboratory.

However, the sera now available from cattle in this herd, serological studies will be continued to determine the relationship, if any, of the viruses isolated from these cattle to squamous cell cancinoma.

Serial No. NIAID-68
1. Infectious Diseases
2. Virus and Rickettsial
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Studies of Tumor Viruses in Nature

Principal Investigators:	Dr. Robert J. Huebner & Dr. Wallace P. Rowe
Other Investigators:	Laboratory - Dr. Janet W. Hartley & Mrs. Joan Austin Field - Mr. William T. Lane and Mr. Jack Reynolds
Cooperating Units:	Dr. Davis Johnson, Smithsonian Institute Dr. E. Baker, Smithsonian Institute Dr. G. L. McKee, Agricultural Marketing Service US Department of Agriculture Dr. Donald Armstrong, Mr. Jack Reynolds, Dr. Melahut Okuyan and Dr. Robert J. Lehman, National Cancer Institute

Man Years: (calendar year 1961)

Total:	57/12
Professional:	4/12
Other:	53/12

Project Description:

Objectives: This project is concerned with elucidating the occurence and behavior of tumor viruses in their natural animal hosts in natural environments. Most of our attention is directed to those viruses for which survey tools have been developed, namely, polyoma in mice; papilloma in rabbits; and Rous sarcoma and avian leukosis complex, in chickens. Because they frequently becloud the issue and lead to confusing data, other naturally occurring viruses of mice and chickens must be studied as well.

Methods Employed: Survey tools - direct virus demonstration and isolation techniques plus serologic procedures (CF, HI, MAP, Neut. tests) are evaluated and used in examining specimens collected in field studies for evidence of contemporary and prior virus infections.

Part B included: Yes

Field Studies:

Polyoma: The basic natural cycle of polyoma infection in the house mouse (*Mus musculus*) appears to exist primarily in the rural agricultural ecology. The largest, most dense and most stable populations of *Mus* are found in relation to grain and hay storage areas on the farm, in grain and feed mills and in regional grain storage depots.

During studies that continued for the entire year polyoma infection of mice was demonstrated in mice on 4 of 15 Maryland farms, 6 of 6 Maryland grain and feed mills and 3 of 4 large Indiana mills with large depot storages. Polyoma infection was demonstrated serologically and also by virus isolations in urine and tissues of wild mice and by tests of nesting materials.

Polyoma contamination of storage grains (corn, oats, wheat) and mixed livestock feeds was demonstrated in 50 per cent of over 60 grain specimens detected because they showed visual evidence of mouse droppings. Virus was isolated only from those specimens taken from grain storage areas harboring mice that were serologically positive (HI) for polyoma.

Factors in Spread: These appear to be similar in both urban and rural ecologies. They include the extreme hardiness of polyoma virus, the communal nest, the demonstrable extensive contamination of nesting areas, chiefly by urine, and the large and dense populations of comparatively young mice (our retrapping program indicates that wild commensal mice seldom, if ever, live longer than 6 months), which insures a regular supply of new susceptibles. The agricultural grain economy in all its various aspects (at least in the eastern part of the United States) apparently supports a heavy infestation of house mice which frequently live and/or feed in stored grain supplies.

The extent of infestation in the United States was indicated by a 29-state survey during the early 1950's which showed that 20 per cent of wheat and 95 per cent of corn specimens examined contained visual evidence of mouse droppings. While mouse droppings can frequently be separated from grain it provides a minimum index of the presence of urine, the chief source of polyoma virus which cannot be eliminated by separatory screens or detected by visual examination. The extent of mouse contamination was indicated best in corn storage specimens which revealed a national yearly average count of 80 mouse pellets per peck of corn. Reports from England in the 1940's revealed extensive infestation, damage and contamination of corn and wheat ricks by *Mus musculus*, thus suggesting widespread infestation paralleling that reported in ancient Egyptian, Greek and Biblical literature published as early as [illegible] B.C.

Other Viruses in Grain Storage Mice: Lymphocytic choriomeningitis, mouse reoviruses, K virus, TA virus, Theiler's virus, mouse hepatitis viruses, and salivary gland virus, have been found in the rural wild mice. There is little doubt that additional viruses will be found when we devise tests for them, namely, mouse leukemia, mammary cancer virus and others. We have not yet tested for these viruses in grain specimens, but this represent one of our future objectives.

Rabbit Papilloma: This project, a survey for tumors and CF antibodies in Kansas and Maryland cottontails, has been suspended since Dr. David White returned to Australia. It has been replaced by a resumption of laboratory studies aimed at growing the virus in tissue culture, and studies of the apparent biological relationships between polyoma, papilloma, K virus, Kilham's rat virus, and the SV 40 virus of monkeys.

Chicken Cancer Viruses: Rous sarcoma, avian lymphomatosis and myeloblastosis just beginning and are very much in the development stage in our laboratory. We must still acquire suitable "SPF" facilities for handling young chickens and set up a virus-defined "Gal"-free and lymphomatosis-free, but susceptible strain of chickens. The latter, a 15 I strain has been located in the agricultural experimental station in Alberta, Canada, will soon be in production at the Damascus Farm of Microbiological Associates, Inc., Bethesda.

In the meantime, Drs. Armstrong and Okuyan are attempting to develop in vitro tests for the avian viruses which will be suitable for survey purposes.

Significance and Proposed Course of this Project:

These studies stress rather uniquely a biological and natural history approach to the study of cancer viruses. They are unique because most other studies of cancer viruses are concerned with basic virus-cell relationships and clinico-pathological approaches.

The findings on polyoma and other viruses in relation to grain supplies raises a number of interesting questions, the most important of which are their significance for laboratory mice and domestic livestock which consume feed grains in the raw state and for man who commonly cooks his grain but nevertheless is widely and frequently exposed to raw grain and flour.

It seems likely that extensive and persistent polyoma infection of grain feeds could easily explain the widespread infection of both commensal and laboratory mice, since grain and grain products represent the major staple of their diet. Studies of possible polyoma infection of livestock and of man has only recently begun. We are anxious to test the most heavily exposed populations not only for antibodies to polyoma, but for other mouse viruses as well. Our initial efforts (under Dr. Armstrong) will be focused on grain and feed mill workers. Naturally, we must define the spectrum of virus infections in the mouse colonies of mills surveyed.

Infection of man with polyoma seems unlikely on the basis of our present evidence. Inhibitors of the polyoma HA are not infrequently found in low titer in human sera, but the specificity of such reactions can be questioned since we have never demonstrated conclusively the presence of neutralizing or CF antibodies. Serums from exposed mill workers, patients with warts (caused by a biologically similar virus) and from persons of various ages will be examined for antibodies to a variety of human and animal viruses. One interesting point would be to determine the presistence of the polyoma inhibitor in human serums.

Only a few bovine serums have been tested, and serums from other grain-eating livestock have not been tested at all. However, Dr. Lehman with the assistance of local practicing veterinarins has been charged with the responsibility for obtaining specimens from cattle, sheep, swine and chickens, specimens suitable for testing not onlyfor polyoma but also for other virus infections particularly avian sarcoma and leukosis.

Future Expansion of Ecology Studies of polyoma, other mouse viruses and subsequently of chicken viruses will be carried out by Mr. Jack Reynolds and a field staff yet to be acquired. This study is awaiting the construction of field facilities in Hagerstown, and the acquisition of additional supplies and transportation.

Sero-epidemiologic studies of human cancer are contemplated for the future, and viral surveys such as that of mill workers will be mounted soon. However, systematic surveys will probably not be feasible for at least a year for the following reasons: Before this study can properly begin, we must have a well planned program based on the availability of serological reagents (not yet contracted for), a sound and feasible questionnaire, and a well indoctrinated effective field team which comprehends fully the possibilities and the objectives of the study. All of this must be achieved before tests can be carried out in the laboratory. In order to do the latter we must first complete new facilities, acquire large supplies of well evaluated diagnostic reagents and new serological personnel who when must be trained.

During 1962 therefore, this project will be concerned with completing field observations on polyoma, but will be most concerned with developing new diagnostic methods, procuring new animal facilities and training personnel for future field studies of additional cancer viruses (especially leukemia). Attempts to determine the importance of viruses in human health in general and in human cancer in particular, will be carried out as soon as we have achieved the necessary trained people and materials. For operating purposes we propose to look very hard at questions implied in the hypothesis that certain human cancers may be zoonotic infections originating in animals.

Part B Included: Yes

Serial No. NIAID-68

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Huebner, R. J.: Cancer as an Infectious disease, Harvey Lecture, 1960-61. In press.

Huebner, R. J.: New "Frontiers" for respiratory virus disease research. Journal of Hygiene, Epidemiology, Microbiology, and Immunology (Czechoslovakia). In press.

Huebner, R. J.: Viruses in search of cancer. Perspectives in virology II (1961). Morris Pollard, Ed., Burgess Publishing Co., Minneapolis, Minnesota.

Huebner, R. J.: New possibilities in virus disease control. Proceedings of the American Life Convention, 1960, In press.

Huebner, R. J., Rowe, W. P., Hartley, J. W. and Lane, W. T.: Mouse polyoma in a rural ecology. CIBA Foundations Reports, In Press.

Honors and Awards - Dr. Robert J. Huebner

Paper - "New Development in Cancer-Virus Research" - The University of Oklahoma Medical Center, Oklahoma City, Oklahoma - January 18, 1961

Public Health Seminar - "Viruses and Cancer" , Pittsburgh University, Pittsburgh, Pennsylvania, February 22, 1961.

Paper - "Virus Infections and Respiratory Disease" - U.S.P.H.S. Hospital, Brighton, Massachusetts, March 22, 1961.

Attended Conference on Science Information, sponsored by AIBS and Council for Advancement of Science Writing - Princeton, New Jersey, March 5,6,&7, 1961.

Chairman of symposium "Role of Viruses in Respiratory Tract Diseases" - Academy of Pediatrics, Washington, D.C., April 10, 1961 (Part of the discussion televised that evening).

Participant in Science Writers' Seminar - sponsored by American Cancer Society, St. Petersburg, Florida, April 10-12, 1961.

Presented paper "New Frontiers for Respiratory Virus Disease Research" at International Conference of Respiratory Diseases - Prague, Czechoslovakia, May 22, 1961.

Presented paper "Morphological Precursors of Cancer" symposium sponsored by CIBA - Perugia, Italy, June 21, 1961.

Participated in Conference on Epidemiology of Leukemia, and presented paper "The Natural History of a Tumor Virus" - Copenhagen, Denmark, September 10-12, 1961.

Participant in Symposium on The Biological Aspects of Viruses in Relation to Medical Problems, and presented paper "Natural History of a Cancer Virus (Polyoma) in Urban and Rural House Mice" - The Jewish Hospital, St. Louis, Missouri, October 13, 1961.

Guest speaker at meeting of ALPHA DELTA KAPPA (International Honorary Sorority for Women Teachers), "Science and Education", Baltimore, Maryland, October 20, 1961.

Paper - "Some Observations on the Natural Histories of Animal Cancer Viruses and Their Possible Implications in Human Cancer" - Univ. Michigan Cancer Research Committee, W. K. Kellogg Gull Lake Biological Station, Michigan.

Guest Lecturer in graduate course in epidemiology - presented paper: "Junior Village Studies", University of California, Berkeley, California. November 1-2, 1961.

Paper: "The Problems and Implications of Animal Cancer Viruses" - Association of Military Surgeons - Washington, D.C., November 7, 1961.

Serial No. NIAID 68 A
1. Infectious Diseases
2. Virus and Rickettsial
3. Bethesda, Maryland

PHS - NIH
Individual Progress Report
Calendar Year 1961

Part A.

Project Title: Sero-epidemiology of virus infections.

Principal Investigator: Dr. John L. Sever, Dr. Robert J. Huebner

Other Investigators: Anita Ley, Flora Wolman, Renee Traub, Horace Turner

Cooperating Units: NINDB, Collaborative study on cerebral palsy, mental retradation, and other neurological and sensory disorders of infancy and childhood (Dr. Richard Masland and R. Hienz Berendes).

MBA - Dr. Gabriel Castellano

Man Years (calendar year 1961):
Total: 180/12
Professional: 48/12
Other: 132/12

Project Description:

Objectives: To utilize serologic techniques in an intensive study of newly recognized viruses as to their relation to the incidence of birth defects, acute undifferentiated respiratory diseases, chronic diseases, and cancer. To develop, wherever technically possible, the refinements of serologic methods necessary for a large scale investigation of the natural course of the disease as caused by viral infections.

Methods Employed: Serologic techniques, including complement-fixation tests, hemagglutination-inhibition, hemadsorption, viral neutralization, and tissue culture neutralization tests are now being developed for the identification of over 100 viral infections.

A large number of serial bleedings are being obtained from mothers during the course of pregnancy and from abnormal infants four months after birth in the Collaborative Study on Cerebral Palsy, Mental Retardation, and Other Neurological and Sensory Disorders of Infancy and Childhood of NINDB. These sera are now being tested for human sera for viral serologic analysis and identification of previous viral experiences are available from studies of

Part B included: Yes

common acute undifferentiated respiratory diseases, and various detailed studies of special virus disease problems of current interest to the Laboratory of Infectious Diseases.

The commercial production and standardization of antigens and antisera suitable for the performance of the serological tests is being utilized. The present program includes further development of integrated laboratory facilities, employing trained technicians capable of handling large scale testing.

Major Findings: Complement-fixing antigens for more than 80 viruses have now been prepared by Microbiological Associates, Inc., in consultation with the Virology Section, LID and NINDB. The antigens which have been prepared are titered in both laboratories and when found acceptable are produced in quantities of 100 to 1000 ml. These include complement-fixing antigens for: Adenoviruses (common antigen), Coxsackie A and B viruses (30 types), Influenza A, B, C, mumps, Parainfluenza (4 types), Polioviruses (3 types), ECHO viruses (28 ytpes), measles, and Herpes Simplex and Respiratory syncytial. Viral antigens for hemagglutination tests have also been produced for 28 adenoviruses, 4 parainfluenza viruses, 3 Reoviruses, mumps, and other viruses which are utilized in routine tests in our laboratories. Greater quantities of satisfactory reagents are now being prepared.

A large scale program for producing specific immune antisera in human volunteers at the Petersburg Federal Reformatory has been initiated and will be pursued during the next calendar year. After the completion of safety tests, groups of volunteers will be given purified viral antigens. The pedigreed stock antisera obtained in this way will be standardized for evaluating the potency of antigen preparations.

The application of a new micro technique to hemagglutination, complement-fixation, and metabolic inhibition tests will permit the use of considerably less sera and antigen for the performance of the various tests. The technique has now been developed to the point where it may be applied in lieu of standard techniques. The Cooke Engineering Company of Alexandria, Virginia, has recently produced and placed on the market all of the refined and developed equipment reported in our publication (Part B). Eight times as many tests may now be performed with the same amount of reagents. The validity of the test procedures have now been established and equipment is being produced to our specifications. The technique has had considerable general acceptance and undoubtedly will become a standard tool for serological investigations.

Preliminary serological investigations of several thousand sera have now been completed. Evaluation of this data in relation to known outcomes of the pregnancies is now under way. Experimental design has mainly consisted of random sampling and matched abnormal-control series. The desirability of the latter approach has been apparent.

Significance to the Program of the Institute: The availability of reliable standard and micro serological techniques for a large group of new viruses provides an opportunity to investigate the course of human diseases caused by viruses which are either difficult to isolate or are resistant to evaluation because the clinical effects are delayed until a long time after infection has subsided. This is particularly true in the case of birth defects and animal cancers. The application of this tool for analysis should provide considerable information on the epidemiological aspects of virus infections.

The program conducted in association with the NINDB should provide the tools necessary to help establish the clinical importance of over 120 new viruses of man.

Proposed Course of the Project: During the year 1962, the serological program will be expanded both in terms of antigenic materials and space for the performance of an increased testing program. The space available has already been increased and will be enlarged. The continuing process of evaluating new antigens and the volume production of antigens will be continued. The evaluation of serial specimens from the NINDB study will proceed in conjunction with information on birth defects being supplied by the Collaborating Institutions. Special investigations will be conducted using pregnant animals and various live virus preparations.

Serial No. NIAID 69 A
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Study of non-bacterial respiratory disease A) Children.

Principal Investigator: Dr. Robert M. Chanock

Other Investigators: Dr. K. M. Johnson, Dr. R. J. Huebner

Cooperating Units: Dr. R. H. Parrott, Children's Hospital Research Foundation, Washington, D. C.

Man Years (calendar year 1961):

Total:	30/12
Professional:	5/12
Other:	25/12

Project Description:

Objectives: 1) To search for new agents responsible for respiratory illness of infancy and childhood. 2) To delineate the epidemiology of viruses which have recently been associated with respiratory illness. 3) To continue the study of certain well established respiratory viruses, i.e., their epidemiology and contribution to the overall respiratory disease experience during infancy and childhood. 4) To determine how much pediatric respiratory illness can be associated with known viruses as a guide to future immunoprophylaxis.

Methods Employed: Infants and children with respiratory illness and suitable controls without such illness will be studied at Children's Hospital District of Columbia. The severe lower respiratory syndromes will be investigated in hospitalized patients, while the milder febrile respiratory disease syndromes will be studied among clinical patients.

The main emphasis will be on the use of various tissue culture systems and the fluorescent antibody technique for virus isolation. An attempt will be made to inoculate specimens from patients directly into tissue culture without prior freezing and thawing, since such treatment appears to rapidly inactivate respiratory syncytial virus and the current strains of influenza B. Fluorescent antibody techniques will be applied to the search for agents which may possibly grow in tissue culture without causing cell destructive effects.

After the various isolates are identified, their contribution to the different respiratory disease syndromes will be estimated by comparing the

recovery rate in such groups with that observed for healthy children free of respiratory symptoms. Children with severe illness admitted to the hospital, as well as control subjects, will be studied serologically for evidence of infection with 15-20 known respiratory viruses as well as any new agent which may emerge as a potentially important pathogen.

Major Findings:

Respiratory syncytial (RS) virus: During the winter of 1960-61, 94 strains of RS virus were recovered from infants and children. Ninety-three strains were recovered from patients with respiratory illness while only 1 strain was isolated from a large group of control subjects. The virus was recovered most often from small infants with bronchiolitis or pneumonia (37%). These findings confirm those of 1959-60 and emphasize the importance of this agent in serious respiratory disease occurring early in life. Indeed, 3 of the small infants who were virus positive died within 1 to 3 days after admission to the hospital.

Analysis of the serologic findings for the past 4 years indicates that RS infection was associated with approximately 20% of all hospitalizable pediatric respiratory disease. RS virus has been disseminated extensively in the pediatrics population of the Washington, D. C. area during each of the past 4 years. Outbreaks of infection have been circumscribed, lasting usually 3 to 5 months. It appears that this virus constitutes a yearly threat to infants and small children.

Para influenza viruses: Para influenza viruses have continued to play an important role in all types of pediatirc respiratory disease. Types 1 and 3 were active in infants and children during most months of the year; during the past 48 months, type 3 virus has been recovered during all but 4 months. Twenty isolations of para influenza type 2 during the past year, predominantly from patients with respiratory disease, confirm the association of this agent with respiratory illness. Unlike the types 1 and 3, the type 2 virus appears to disseminate in a more sporadic fashion.

Adenoviruses: During the last 4 years approximately 500 strains have been recovered from infants and children. Analysis of virus recovery data by type indicates that types 1, 3, 5 and 7 were definitely producing disease. A disease association could not be established for type 2. Fifty of the strains recovered belonged to the higher types above type 7; all of these higher types were recovered only from anal swabs and never from throat specimens.

Enteroviruses: During July, August and September, 1961 35 strains of Coxsackie B5 were recovered. This virus was isolated 8 times more often from children with respiratory disease than from controls. B5 virus infection was associated with febrile upper respiratory disease which was indistinguishable from that associated with myxovirus or RS infection.

The role of the newly recognized fastidious enteroviruses in childhood respiratory disease has been under scrutiny. Attempts to recover these agents in human diploid tissue cultures were made with specimens from 250 children who had upper respiratory illness over the past 3 years as well as with throat swab material from 250 matched controls. Eleven strains (4.4%) were recovered from the former group while only 3 strains (1.2%) were isolated from the latter group. In this limited study an association of these agents with illness was suggested but the isolation rate was too low to permit definitive conclusions to be reached. It is surprising that these viruses are recovered so seldom from children considering their prevalence in adults.

Significance to the Program of the Institute: Respiratory disease is the most common infectious ailment of man. Such infections occur commonly and in their severest form during infancy and early childhood. Detailed knowledge of the agents responsible for respiratory disease and their nautral history are a necessary prelude to attempts at either immunoprophylaxis or chemotherapy. As has been shown with the respiratory syncytial and para influenza viruses, the significance to be derived from newer respiratory agents discovered in childhood illness is also increased because they are associated with infections which may produce a generally milder illness in adults.

Proposed Course of the Project: It is planned that this study will continue for a period of several years, since a large segment of childhood respiratory illness remains to be elucidated. In addition, viruses responsible for respiratory illness vary at different times and in different localities. Therefore, surveillance should continue in order to comprehend the larger picture. The role of the fastidious enteroviruses in childhood respiratory disease will be vigorously pursued by testing all specimens from the Children's Hospital study in human diploid tissue cultures on a current basis.

Part B Included: Yes

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Chanock, R. M., Bell, J. A., and Parrott, R. H.: Natural history of para influenza infection. Monograph in: PERSPECTIVES IN VIROLOGY II: 126-138, 1961, M. Pollard, Ed.

Chanock, R. M., Kim, H. W., Vargosko, A. J., Deleva, A., Johnson, K. M., Cumming, C., Parrott, R. H.: Respiratory syncytial virus. I. Virus recovery and other observations during 1960 outbreak of bronchiolitis, pneumonia, and minor respiratory disease in children. J.A.M.A. 176: 647-653, 1961.

Parrott, R. H., Vargosko, A. J., Kim, H. W., Cumming, C., Turner, H. C., Huebner, R. J., Chanock, R. M.: Respiratory syncytial virus. II. Serologic studies over a 34-month period of children with bronchiolitis pneumonia, and minor respiratory diseases, J.A.M.A. 176: 653-657, 1961.

Kim, H. W., Vargosko, A. J., Chanock, R. M., Parrott, R. H.: Para influenza 2 (CA) virus: etiologic association with croup. Pediatrics 28: 614-621, 1961.

Honors and Awards relating to this project.

1. Member of U.S. delegation on Virology which visited the USSR, April and May 1961.

2. Invited speaker at 1st International Congress on Respiratory Tract Diseases of Virus and Rickettsial Origin, Prague, May 1961.

3. Member of the virus study group of W.H.O. to advise on viral reference reagents, virus nomenclature, and the role of W.H.O. in virus research, August 1961.

4. Invited to lecture at 3rd Symposium of Perspectives in Virology, Feb. 1962.

5. Participant in Symposium on Viral Respiratory Diseases, American Public Health Associati on Annual Meeting at Detroit, November 1961.

6. Invited to lecture at Boerhaave Postgraduate Course on Respiratory Viral Diseases, Leiden, Holland, April 1962.

7. Invited to participate in Symposium on Viral Respiratory Diseases, New York Academy of Medicine, February 1962.

8. Presented paper on "Ecology of Eaton Agent and Cold Agglutinen Positve Atypical Pneumonia" at the American Epidemiological Society, April 1961.

9. Co-author on "A Manual of New Viruses Causing Respiratory Illness in Man", Prepared for the Commission on Acute Respiratory Diseases of the Armed Forces Epidemiology Board, 1961.

Serial No. NIAID 69 B
1. Infectious Diseases
2. Virus and Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Study of Non-Bacterial Respiratory Disease
B) Adults: Military populations.

Principal Investigator(s): Dr. Karl M. Johnson, Dr. Robert Chanock

Other Investigators: Dr. Robert J. Huebner, Dr. Maurice A. Mufson, Dr. A. L. Kisch

Cooperating Units: Dr. H. H. Bloom, Dr. B. Forsythe, Naval Medical Field Research Laboratory, Camp Lejeune, N. C.

Man Years (calendar year 1961):

Total:	30/12
Professional:	5/12
Other:	25/12

Project Description:

Objectives: To study on a continuing basis the role of newly recognized viruses in adult respiratory illness. This study was organized in order to compare viral experience of men fresh from recruit training with that of individuals forming the stable cadre personnel. An opportunity is also provided to assay the effectiveness of certain viral vaccines administered to personnel during their recruit training at another military center. The study is also being expanded to include men in recruit training at Parris Island, South Carolina.

Methods Employed: Clinical records and appropriate specimens are collected routinely from individuals in various dispensaries at Camp Lejeune, North Carolina. Such materials are also obtained from individuals hospitalized at this base, and in certain instances, from children, wives, and mothers seen in dependent outpatient clinics. Special emphasis has been placed upon the collection of specimens from matched control groups.

Clinical specimens are tested in monkey kidney, HEp-2 and diploid human embryonic tissue cultures. Certain other tissue cultures are employed whenever indicated. Paired serum samples are available from well over 90% of individuals in the study and are assayed for various viral agents. Specimens for virus isolation are divided into two portions and stored in this laboratory and the Camp Lejeune laboratory respectively.

Part B included: Yes

Major Findings:

1. Adenoviruses. [illegible] respiratory disease due to adenoviruses occurred in January-March of 1961. Viruses were predominantly Type 4 with 15-20% Type 7, no Type 3. Continuous study of this camp for more than 2 years has resulted in the finding of an apparent new military epidemiology for adenovirus infection in the United States. Whereas, camps further north seem to have endemic adenovirus infection with constant high basal rates and intermittent peaks, we can now predict an annual epidemic at Camp Lejeune with almost no infection during the remaining 9 months of a year. The 1961 outbreak occurred despite the fact that all men had been given adenovirus vaccine on entry into service.

2. RS virus: Natural infection in adults by RS virus was documented for the first time, and this was shown to represent reinfection, since all subjects were found to have neutralizing antibodies prior to infection.

3. Enteroviruses: Coe virus - cause of an outbreak of respiratory d disease in 1960, was not active in 1961. Using newly described fibroblast cultures of human embryonic origin, however, numerous strains of several new enterovirus-like agents have been recovered - apparently associated with mild cold-like illness.

4. Eaton agent: Definitive evidence for chemotherapeutic control of Eaton agent, atypical pneumonia was obtained in a study at Parris Island. Demethylchlortetracycline (0.9 daily) was shown to shorten fever, arrest progression of radiologic abnormalities, and decrease duration of confinement among persons with Eaton pneumonia, but had no effect in cases caused by adenoviruses. The study was strictly "double-blind" in execution, and some 300 cases were included in the series.

Over an [illegible] period Eaton agent [illegible] to be associated with 56% of the pneumonia illnesses which occurred among recruits. The risk of pneumonia to an incoming recruit at Parris Island remains high (2%).

Significance to the Program of the Institute: This project represents a major continuing inquiry into the nature of respiratory disease in adult [illegible] enterovirus with respiratory disease, as well as providing needed laboratory information concerning para influenza virus infection in adults. A well documented collection of case histories, specimens, and paired sera provides an excellent source [illegible] available for [illegible] of newly discovered agents, as well as those not yet known to us.

Proposed Course of the Project: [illegible] children are now being studied. A series of longitudinal studies of companies is being undertaken at Camp Lejeune (ITR) to learn more of the dynamics of infection by the many viruses which can now be recovered from the respiratory tract of adults.

Parris Island recruit camp will be studied further, in order to integrate this initial phase with the subsequent two parts of the training cycle. In addition, the role of Eaton agent non-bacterial otitis will be investigated. An attempt will be made to recover Eaton agent directly on agar plates by a technique which has proved successful for laboratory adapted strains.

A study of pneumonia among civilian adults will be initiated at the D. C. General Hospital. The possible role of Eaton agent and other respiratory tract pathogens will be assessed.

Part B Included: Yes

Serial No. NIAID 60 B

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Johnson, K. M., Bloom, H. H., Mufson, M. A., and Chanock, R. M.: Acute respiratory disease associated with Coxsackie A-21 virus infection. I. Observations in a recruit population, J.A.M.A. In press.

Bloom, H. H., Johnson, K. M., Mufson, M. A., and Chanock, R. M.: Acute respiratory disease associated with Coxsackie A-21 virus infection. II. Observations in a non-recruit population. J.A.M.A. In press.

Kingston, J. R., Chanock, R. M., Mufson, M. A., Hellman, L. P., James, W. D., Fox, H. H., Manko, M.A., and Boyers, J.: Eaton agent pneumonia. II. Treatment with demethylchlortetracycline. J.A.M.A. 176: 118-123, 1961.

Mufson, M.A., Manko, M.A., Kingston, J.R., Chanock, R.M.: Eaton agent pneumonia. III. Clinical Features, J.A.M.A. 178: 369-374, 1961.

Bloom, H.H., Johnson, K. M., Jacobsen, R., Chanock, R. M.: Recovery of para influenza viruses from adults with upper respiratory illnesses. The Am. J. of Hyg. 74: 50-59, 1961.

Serial No. NIAID 70 A
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Laboratory studies of newly recognized viruses associated with respiratory illness.
A. Eaton agent, para influenza viruses, and respiratory syncytial virus.

Principal Investigator: Dr. Robert M. Chanock

Other Investigators: Dr. K. M. Johnson, Dr. H. V. Coates, Dr. A. L. Kisch, Dr. M. A. Mufson

Cooperating Units: Dr. L. Hayflick, Wistar Institute, Philadelphia, Pa.

Man Years (calendar year 1961):

Total:	20/12
Professional:	10/12
Other:	10/12

Project Description:

Objectives: 1) To investigate the biologic and antigenic properties of the newly recognized parainfluenza viruses and respiratory syncytial virus. 2) To study the properties of Eaton agent and its growth in various types of tissue cultures. 3) To develop simple procedures for the recovery, identification, and serologic study of Eaton agent.

Methods employed: The standard tools of virology - neutralization, complement-fixation, and hemagglutination-inhibition are employed to determine the antigenic specificity and existence of shared antigens among the para influenza viruses.

Enumaertaion of PPLO colonies on agar plates, the fluorescent antibody technique, plus the standard serologic method of complement-fixation are employed in the quantitation and serologic study of Eaton agent and its relationship to other PPLO.

Part B included: Yes

Experimental animals are inoculated with para influenza or respiratory syncytial virus and observed for signs of illness and tested for evidence of viral multiplication. Pathologic anatomy of animals so infected is also studied by standard histopathologic techniques.

Major Findings:

1. Para influenza viruses. Continued study has failed to reveal evidence of antigenic variation within types 1, 2, or 3. This antigenic stability is in contrast to the instability which characterize influenza A and to a lesser extent influenza B.

2. Respiratory syncytial virus. This virus, which is the single most important respiratory pathogen in childhood, has recently been adapted to primary bovine kidney tissue culture. Studies are now underway to characterize such bovine tissue adapted virus and to determine its potential usefullness for immunization of man.

3. Eaton Agent. The Eaton agent was grown on an artificial agar medium. Colonies with typical PPLO morphology developed following inoculation of tissue culture grown Eaton agent onto agar plates. These colonies were further identified as PPLO by their reaction with Dienes strain. Fluorescent antibody tests with paired sera from patients with Eaton pneumonia showed that the colonies were antigenically indistinguishable from the Eaton agent. The Eaton PPLO was found to be antigenically distinct from other human PPLO as well as PPLO recovered from rats, cows, chickens, sewage, and continuous tissue culture cell lines. The agent has also been grown in PPLO broth medium. Preliminary experiments indicate that a complement-fixation antigen can be prepared from a concentrate of broth.

Significance to the Program of the Institute: Knowledge of the antigenic properties of the para influenza viruses is required in order to perform meaningful epidemiologic studies with these agents. Information bearing upon the species specificity of the para influenza viruses greatly adds to our understanding of the natural history of these agents.

Identification of Eaton agent as a PPLO represents an important "first" in infectious disease research. In our previous studies the Eaton agent has been etiologically associated with a considerable proportion of lower respiratory tract illness in children and adults. The finding that the Eaton agent is a PPLO thus represents a definitive identification of a major human respiratory pathogen; it also represents the first etiologic association of a PPLO with human respiratory disease. Since the association of PPLO with urethritis rests on a questionable foundation, the present findings represent the first real association of a PPLO with any type of human disease.

Proposed Course of the Study: Efforts will continue to maintain surveillance of the antigenic properties of the para influenza viruses.

The study of the factors influencing the growth of Eaton PPLO on agar and in broth will be vigorously pursued. An attempt will be made to recover the organism directly on agar plates from infected individuals. The development of a complement-fixation reaction for Eaton PPLO will continue and if successful the technique will be applied to the large number of unsolved problems relating to the natural history of Eaton infection. Finally, the PPLO techniques which ultimately prove to be optimal for Eaton agent will be applied to the search for otherPPLO which may cause respiratory disease in man.

Part B Included: Yes

Serial No. NIAID 70 A

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Chanock, R. M., Hayflick, L., and Barile, M. F., Growth on artificial medium of an agent causing atypical pneumonia and its identification as a PPLO. Proc. Nat. Acad. Sci. In press, 1962.

Kisch, A., Johnson, K. M., Chanock, R. M.: Immunofluorescence with respiratory syncytial virus, Virology In press, 1961.

Serial No. NIAID 70 B
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Laboratory studies of newly recognized viruses associated with respiratory illness.
B) Enteroviruses.

Principal Investigator: Dr. Karl M. Johnson

Other Investigators: Dr. R. M. Chanock, Dr. M. A. Mufson, Dr. A. L. Kisch, Dr. P. Webb, Dr. R. J. Huebner.

Cooperating Units: Dr. L. Rosen, Epidemiology Section, LID

Man Years (calendar year 1961):

Total:	20/12
Professional:	10/12
Other:	10/12

Project Description:

Objectives: To develop simple reproducible methods for laboratory manipulation of enteroviruses, particularly the large group of newly recognized agents which appear to be potential causes of respiratory disease. Studies are designed specifically to determine optimum methods of cultivation of the agents in either tissue culture of small laboratory animals. In addition, efforts are made to A) determine the optimum manner of preparing specific antisera, B) to evaluate various serologic procedures potentially of use in field studies, and C) since many new viruses are fastidious in their growth requirements, to study where indicated certain fundamental properties of these viruses.

Methods Employed: These will be described where pertinent under consideration of results so far obtained with certain of the viruses.

Major Findings:

Coxsackie A-21 virus: A hemagglutinin for human "O" cells has been demonstrated. A useful serologic test based on this property has been worked out. It has been demonstrated that two types of virus particle are present in early tissue culture harvests - one that hemagglutinates (HA+) and one that does not (HA-). Growth of the mixture in malignant cell culture selects for the HA- variant, whereas primary cell cultures appear to support both types equally well. Various implications of these observations are being pursued.

It has also been found that cytopathic effects (CPE) are less readily demonstrated in malignant cells incubated stationary as compared to rotated. Viral multiplication, however, was about equal under both conditions.

Tissue culture strains virus produced paralysis in suckling mice but large amounts of virus were necessary to demonstrate this effect.

New Enteroviruses: Several new potential respiratory pathogens have been characterized as enteroviruses. The agents have been adapted to growth in semi-continuous human fibroblasts and in malignant human tissues (KB). Problems in preparing high-titered antisera for purposes of identification of virus strains are under study. Use of KB grown virus, adjuvants and guinea pigs has given promising early results.

Significance to the Program of the Institute: Enteroviruses promise to play an important role in human respiratory disease. Before significant epidemiologic progress can be made, however, major advances in understanding the biologic properties of the newer types are needed. In the course of these studies opportunities for genetic studies which may lead to development of strains with different, useful properties are certain to arise. But the primary contribution of the work is likely to be the establishment of laboratory procedures for a standardized approach to studies of the agents in nature.

Proposed Course of the Project: Several additional prototypes are being characterized. Efforts to obtain standard antisera will be continued. Search for hemagglutinating and complement-fixing antigens is being undertaken.

Part B Included: Yes

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project;

Johnson, K. M., Bloom, H. H., Rosen, L, Mufson, M. A., Chanock, R. M.: Hemagglutination by Coe virus. Virology 13: 373-374, 1961.

Honors and Awards Relating to the Project:

Presentation of paper entitled "Enteroviruses" at the Annual Meeting of the American Public Health Association, November 13, 1961, Detroit, Michigan.

Invited to lecture N. Y. Academy of Medicine, February, 1962.

Participant: Boerhaave Post-Graduate Course on Respiratory Viral Infections Leiden, Holland, April 1962.

Invited to lecture Dartmouth Medical School, Hanover, New Hampshire, November 30, 1961.

Presentation of paper entitled "Epidemic of Coe Viruses (Coxsackie A-21) in Military Recruits" at the Annual Meeting of the American Epidemiological Society, April 1961, Atlanta, Georgia.

Co-author on "A Manual of New Viruses Causing Respiratory Illnesses in Man" Prepared for the Commission of Acute Respiratory Diseases of the Armed Forces Epidemiological Board, 1961.

Serial No. NIAID-71
1. Infectious Diseases
2. Virus & Rickettsial
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Studies of tumor-producing viruses.

Principal Investigator: Dr. Wallace Rowe, Dr. J. W. Hartley

Other Investigators: Dr. Robert J. Huebner, Dr. Okuyan, Dr. Donald Armstrong, Mr. L. W. Smith

Cooperating Units: Dr. L. W. Law and C. J. Dawe, NCI

Man Years (calendar year 1961):

Total: 29/12

Professional: 10/12

Other: 19/12

Project Description:

Objectives: To characterize tumor viruses of mice and other species from the standpoints of development of laboratory procedures for rapid recognition of the virus and antibodies against the virus, their taxonomy, general properties, and epidemiology.

Methods Employed: Tissue culture, animal inoculation, egg inoculation, and interference assays for demonstration and standard serological procedures.

Major Findings:

A. Mouse polyoma virus. Work with this agent is detailed under project Number 68 .

B. Mouse leukemia viruses. The Moloney mouse leukemia virus has been successfully propagated in tissue cultures both as explant cultures of leukemic tissues and in cultures of mouse embryo. Extensive attempts were made to develop an interference assay in infant mice for rapid recognition of the agent. Initial results were positive, but as detailed in Project Report 71B, the positive results were eventually shown to be due to contamination by the LDH virus.

Part B Included: Yes

C. The monkey vacuolating virus. Because of the demonstration by Dr. Bernice Eddy that this agent is tumorigenic for hamsters, preliminary studies were made of its growth cycle in hamsters and of some of its laboratory properties. Surprisingly, there was no evidence of propagation of the virus in suckling hamsters during the first four weeks after inoculation, although several recipients have subsequently developed tumors. These results suggest that its pathogenic pattern for hamsters may be more like that of papilloma virus, where the virus only proliferates as the tumor develops, rather than like polyoma virus, in which the virus proliferation precedes the tumor formation by a long interval. We have assisted Dr. Clyde Dawe in carrying out organ culture studies to ascertain possible in vitro tumor formation in human tissue by the vacuolating virus. It was found that the virus persists and almost certainly multiplies in human embryonic kidney and salivary gland organ cultures for periods of as long as 70 days. Suggestive evidence of proliferative stimulas by the virus was obtained by Dr. Dawe and this finding is being further explored by him in collaboration with our group.

Significance to the Program of the Institute: The development of reliable laboratory procedures for recognition of infection with animal tumor viruses is the most potentially important contribution that could be made to tumor virology at the present time. Both the understanding of the natural history of tumor virus infections and delineation of mechanisms of tumorigenesis are almost totally dependent on the techniques available for the handling and recognition of the tumor viruses. Also, any reliable technique developed for animal tumor viruses offers that much more possibility for applying the findings with the animal tumor viruses to the problem of human cancers.

Proposed Course of the Project: Two areas will be particularly emphasized in the coming year - tissue culture studies of the mouse leukemia viruses and the adaptation of recently described laboratory procedures for avian tumor viruses for epidemiological studies. With the mouse leukemia viruses, studies will be primarily directed toward developing procedures for production of high titer tissue culture grown virus and for recognition of presence of infection in tissue cultures by rapid in vitro procedures, such as interferon production, hemagglutination, or enzymatic changes in the tissue cultures. With the chicken tumor viruses, assays for the Rous interfering factor (RIF) and for antibodies to Rous sarcoma will be developed and epidemiologic studies in chicken flocks will be initiated. Studies will also be made for the possible occurrence of neutralizing antibodies to avian tumor viruses in individuals of other species. The studies of the vacuolating virus - hamster system will be extended.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Rowe, W. P., Huebner, R. J., and Hartley, J. W.: The ecology of a mouse tumor virus. Perspectives in Virology II: 177-190, 1961.

Rowe, W. P.: The epidemiology of mouse polyoma virus infection. Bacteriological Reviews 25: 18, 1961.

Rowe, W. P., Hartley, J. W., and Huebner, R. J.: The natural history of polyoma virus infection - a summary. Canadian Cancer Conference. Academic Press, Inc. 4: 271-276, 1961.

Dawe, C. J., Law, L. W., and Rowe, W. P.: Influence of age, species and immune factors on the response of salivary gland to polyoma virus in tissue culture. Pathologie et Biologie 9: 711-718, 1961.

Rowe, W. P.; Mouse polyoma virus infection. Trans. and Studies of Coll. of Phys. of Phila. 29: 66-70, 1961.

Honors and Awards relating to this project:

Mary Scott Newbold lectures, Philadelphia Academy of Medicine, March 1961.

Invited speaker, Francis Delafield Hospital, September 30, 1961.

Invited speaker, Symposium in Tumor Viruses, Mass. General Hospital, October 16, 1961.

Serial No. NIAID- 71B
1. Infectious Diseases
2. Virus & Rickettsial
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Studies of mouse viruses with special reference to their importance as extraneous viruses ("background noise") in mouse tumor systems.

Principal Investigator: Dr. Wallace P. Rowe and Dr. Janet W. Hartley

Other Investigators: Dr. Robert J. Huebner, Dr. Dietrich Falke, Dr. Paul H. Black

Collaborating Units: Microbiological Associates Research Division - Dr. Thomas Ward

Man Years (calendar year 1961):

Total: 123/12

Professional 35/12

Other 88/12

Project Description:

Objectives: To characterize the viral flora of the mouse with particular reference to developing procedures for recognition of infection; to determine the epidemiological patterns of the various mouse viruses for the purpose of defining the problem of extrinsic viruses as complicating factors in studies of mouse tumor viruses.

Methods Employed: Serologic, tissue culture, and animal pathogenicity studies. Attempts to isolate agents from laboratory and wild mice and from mouse tumors. Studies of the epidemiology of the agents under natural and artificial conditions.

Major Findings: Extensive sero-epidemiologic studies of those mouse viruses for which reliable serologic tests have been developed have been carried out. Satisfactory diagnostic procedures for determining prevalence of infection with a wide variety of mouse viruses have now been developed, so that it is quite feasible to delineate a large portion of the viral flora of mouse colonies by relatively simple and standardized serologic and virus

Part B Included: Yes

detection procedures.

Reoviruses: Antibody to reo 3 virus has been found to be common in stocks of "germ-free mice", but attempts to isolate the virus from germ-free tanks have not yet succeeded. This virus has been found to be a fairly common contaminant of transplanted mouse tumors in at least one laboratory. It has been found to be a fairly common contaminant of transplanted mouse tumors in at least one laboratory. It has been found that suckling mouse brain provides an excellent source of complement-fixing antigen for reo 3 virus. Studies of the specificity of the soluble and viral CF antigens from mouse brain have shown that both of these antigens are group-specific in their reactivity, as contrasted with the majority of virus groups in which the viral antigen is type-specific. Hemagglutination inhibition procedures for serologic differentiation of reo 2 viruses into a number of sub-types have been developed. The various reo 2 sub-types have been found to be widely distributed geographically, and among different animal species.

Mouse hepatitis viruses: Particular emphasis has been made on attempting to delineate the mouse hepatitis viruses as a virus group and to develop serologic procedures. Procedures have been developed for production of specific complement-fixing antigens, and a satisfactory neutralization test for several strains has been developed. The various strains of mouse hepatitis virus share a common complement-fixing antigen but there is some type-specificity in their reactivity. Strains of mouse hepatitis have been isolated from several viral and cell induced mouse leukemias. The important finding has been made that mouse hepatitis viruses are readily isolated from the feces of normal mice in infected colonies. This finding, plus the development of serologic procedures, will for the first time permit a meaningful study of the epidemiology of this important mouse virus group. Attempts to activate mouse hepatitis viruses in germ-free mice by passage of *Eperythrozoon coccoides* have been unsuccessful, providing highly suggestive evidence against the transplantal transfer of this virus.

LDH Virus: The LDH virus described by Dr. Vernon Riley of the Sloan Kettering Institute has been found to be identical with the virus discovered here by virtue of its ability to interfer with vesicular stomatitis infection in infant mice. This virus has been characterized to some extent, and has been found to be approximately 60 millimicrons in diameter, ether-sensitive, serologically unrelated to any of the known mouse viruses and to be an extremely poor antigen. It has been found that the LDH agent is a quite frequent contaminant of mouse viruses which have been carried in mouse passage.

Theiler's Encephalomyelitis Viruses: Procedures have been developed for obtaining hemagglutination with all available strains of Theiler's viruses by the use of virus grown in the suckling mouse brain. The Theiler's group has been antigenically characterized with the finding that there are major serological differences between strains, but some sharing of HI antibodies between all strains.

Thymic Agent: This virus has been found to be highly prevalent in wild mice of many different populations, and has now been encountered in

their epidemiology will be continued. Because of the problem of extrinsic mouse viruses contaminating the studies of extrinsic mouse viruses, an effort will be made to develop methods for handling viruses in strict isolation from one another. The use of germ-free isolators for routine virus work will be intensively explored, as well as the value of dispersion of work into separated house trailers. Dr. Rowe is serving as project officer on the contract between the Virus Cancer Panel of the NCI and Microbiological Associates, Inc. for development and production of standard mouse virus identification reagents. This laboratory will certify the reagents produced, thus assisting in the distribution of specific reagents prepared according to the techniques in large part developed here to mouse laboratories throughout the country.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Rowe, W. P., and Capps, W. I.: A new mouse virus causing necrosis of the thymus in newborn mice. J. Exptl. Med. 113: 831, 1961.

Hartley, J. W., Rowe, W. P., and Huebner, R. J.: Recovery of reoviruses from wild and laboratory mice. Proc. Soc. Exper. Biol. & Med. In Press.

Hartley, J. W., Rowe, W. P., Austin, J. B.: Subtype differentiation of reovirus type 2 strains by hemagglutination-inhibition with mouse antisera. Virology. In Press.

Rowe, W. P., Hartley, J. W. Huebner, R. J.: Polyoma and other indigenous mouse viruses. Proceedings of the ICLA Symposium on Diseases of Laboratory Animals, R. J. C. Harris, Editor.

Bell, J. A., Huebner, R. J., Rosen, L., Rowe, W. P., Cole, R. M., Mastrota, F. M., Floyd, T. M., Chanock, R. M., Shvedoff, R. A.: Illness and microbial experiences of nursery children at Junior Village. Am. J. Hyg. In Press.

Kasel, J. A., Rowe, W. P., Nemes, J. L.: Further characterization of the adenovirus erythocyte receptor - modifying factor. J. Exptl. Med. 114: 717-728, 1961.

Honors and Awards relating to this project:

Invited speaker, AVMA Symposium on Diseases of Laboratory Animals, Detroit, August 1961.

Invited speaker, ICLA Symposium on Diseases of Laboratory Animals, Prague, Czechoslovakia, September 1961. (Paper delivered in absentia)

Serial No. NIAID 72 A
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Study of respiratory viruses in human volunteers.
A) Enteroviruses and respiratory syncytial.

Principal Investigator: Dr. Karl M. Johnson, Dr. Robert M. Chanock

Other Investigators: Dr. R. Couch, Dr. V. L. Knight, Dr. H. Evans, Dr. A. Spickard, Dr. J. P. Utz

Cooperating Units: Laboratory of Clinical Investigations, NIAID; Federal Bureau of Prisons

Man Years (calendar year 1961):

Total:	17/12
Professional	5/12
Other:	12/12

Project Description:

Objectives: To determine whether newly recognized viruses of potential respiratory pathogenicity are capable of inducing mild illness in adults under controlled conditions. To examine the experimental pathogenesis of such viral infection, and to provide new information specifically designed to facilitate the study of naturally occurring mild respiratory illness in adults.

Methods Employed: Young male adult volunteers from federal prisons are selected on the basis of a) general good health, and b) neutralizing antibody status for the particular virus to be studied. Volunteers are maintained in groups of three in strict isolation on a clinical service of the NIAID. Histories and routine physical examinations, as well as certain basic laboratory procedures are performed upon admission. Known amounts of test viruses are administered intranasally, and daily clinical observations, as well as appropriate specimens for laboratory analysis, are obtained. Virus specimens are inoculated into appropriate tissue cultures and/or animals. Serologic tests are performed on suitably collected serum samples.

Part B included: Yes

Major Findings:

Respiratory Syncytial Virus: This virus was shown to produce reinfection in adults, with mild illnesses typical of so-called "colds" Infected persons generally shed virus 3-6 days and symptoms averaged 5-6 days in duration. No difference in susceptibility was observed in men having widely different amounts of pre-challenge RS antibody.

Vacuolating (SV_{40}) Virus: This agent, of simian origin, was administered to adults in the monkey kidney grown RS virus pool prior to its recognition and description by Sweet and Hilleman. In a subsequent experiment the RS virus was pre-neutralized, and SV_{40} given intranasally. No acute illness was observed, although virus was recovered from 2 men and a majority showed serologic responses; this finding represents the first documented evidence that this common simian virus is capable of human infection.

Coxsackie A-21 Virus: Volunteer studies with this virus have proved important biological information concerning the first enterovirus epidemiologically linked with respiratory disease.

Subjects without A-21 neutralizing antibodies were readily infected intranasally with small amounts of virus. Acute respiratory illness, often febrile with myalgia and headache, wer often seen 2-3 days after challenge. Virus was usually recovered from throat swabs from such persons for 2-3 weeks and in some for as long as 40 days. Fecal excretion was more sporadic and generally of lesser duration. Antibody responses occurred between 13 and 18 days after challenge - long after all symptoms had disappeared. Subjects with high antibody levels generally could not be infected, and those with intermediate levels usually had infections marked by shorter virus excretion, more rapid serologic response and absence of clinical illness.

Studies with virus administered in an enteric coated capsule indicated that susceptible adults were extremely difficult to infect via the gastrointestinal route. The data strongly suggest that this virus is a respiratory pathogen, spread, at least in adults, via the respiratory rather than the enteric tract.

Significance to the Program of the Institute: This project has resulted in the experimental demonstration of a significant new concept concerning the pathogenesis of mild respiratory illness in adults. Many such illness may thus represent reinfection with an agent which caused severe illness at an earlier time. The project has also provided valuable information pertinent to the design and execution of future field studies of RS virus infection. The Coxsackie A-21 experiments showed that a virus with all the biophysical properties of an enterovirus can nevertheless be restricted primarily to the upper respiratory tract of man.

Proposed Course of the Project: Several new enteroviruses will be studied in efforts to obtain biologic information of great utility to subsequent field studies of these agents. Serologic analyses will be made in efforts to learn more of the inter-relationships between different agents. Problems of clinical as well as serologic immunity will be studied through rechallenge experiments. It is also hoped that certain virus-host variables can be manipulated (dosage, aerosol particle size, temperature, humidity) in order to learn more concerning the dynamics of such human infection.

Part B Included: Yes

Serial No. NIAID 72 A

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Kravetz, H. M., Knight, V., Chanock, R. M., Morris, J. A., Johnson, K. M, Rifkind, D., and Utz, J. P.: Respiratory Syncytial Virus. III. Production of illness and clinical observations in adult volunteers, J.A.M.A. 176: 657-663, 1961.

Johnson, K. M., Chanock, R. M., Rifkind, D., Kravetz, H. M., and Knight, V.: Respiratory syncytial virus. IV. Correlation of virus shedding, serologic response, and illness in adult volunteers. J.A.M.A. 176: 663-667, 1961.

Morris, J. A., Johnson, K. M., Aulisio, C. G., Chanock, R. M., and Knight, V.: Clinical and serologic responses in volunteers given vacuolating virus (SV_{40}) by respiratory route. Proc. Soc. Exptl. Biol. Med. 108: 56-59, 1961.

Serial No. NIAID 72 B

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Study of respiratory viruses in human volunteers, B) Eaton agent and adenoviruses.

Principal Investigator: Dr. Robert M. Chanock, Dr. Karl M. Johnson

Other Investigators: Dr. R. Couch, Dr. V. L. Knight, Dr. H. Evans, Dr. A. Spickard, Dr. J. P. Utz

Cooperating Units: Laboratory of Clinical Investigations, NIAID

Man Years (calendar year 1961):

Total:	12/12
Professional:	5/12
Other:	12/12

Project Description:

Objectives: To determine whether newly recognized viruses of potential respiratory pathogenicity are capable of inducing mild illness in adults under controlled conditions. To examine the experimental pathogenesis of such viral infection, and to provide new information specifically designed to facilitate the study of naturally occurring mild respiratory illness and pneumonia in adults.

Methods Employed: Young adult male volunteers from federal prisons are selected on the basis of a) general good health, and b) neutralizing antibody status for the particular virus oragent to be studied. Volunteers are maintained in groups of three in strict isolation on a clinical service of the NIAID. Histories and routine physical examinations, as well as certain basic laboratory procedures are performed upon admission. Known amounts of the test agent are administered intranasally, and daily clinical observations, as well as appropriate specimens for laboratory analysis, are obtained. Specimens are inoculated into appropriate tissue cultures and/or animals. Serologic tests are performed on suitably collected serum samples.

Major Findings:

Eaton Agent: A recently isolated tissue culture strain of Eaton agent infected all 27 volunteers who were free of antibody prior to challenge. In addition 17 of 25 men who possessed fluorescent stainable antibody titers of 1:10 or greater were infected. Three pneumonia illnesses developed in the

Part B included: Yes

former group. In addition, 11 men in this group developed tympanitis and 2 others had a febrile upper respiratory illness. Illness with physical findings in the sero-positive group was limited to one volunteer who developed tympanitis. The aggregation of illness predominantly in the group free of antibody at the time of challenge indicated that the Eaton agent was responsible for the pneumonia, tympanitis, and other febrile illness which occurred.

Adenovirus: Second human kidney tissue culture passage Type 7 adenovirus was given in an enteric coated capsule to 9 volunteers. By this procedure virus was not released until the capsule reached the lower bowel. Illness did not occur. Two of the volunteers were free of detectable neutralizing antibody prior to virus challenge. Both men excreted virus and developed neutralizing antibody. Virus was recovered from the anal specimens but never from the throat.

Significance to the Program of the Institute: The production of respiratory tract disease in the Eaton volunteer study provided the final proof that this agent is a respiratory pathogen. This finding is of considerable significance in view of the controversial history of the Eaton agent. The occurrence of ear disease in the Eaton volunteers has provided the first clue to the etiology of naturally occurring non-bacterial middle ear disease. Now that we know that Eaton agent is a PPLO its predilection for the middle ear is not surprising since many animal PPLO have a similar tropism.

The preliminary findings from the adenovirus volunteer study suggest that it may be possible to immunize against Type 7 by setting up a limited infection in the lower gastro-intestinal tract. In this manner the upper respiratory tract, which is the site of pathology in nautral infection, would be by-passed. Successful live virus immunization with adenovirus Type 7 would have important implications for the prevention of childhood and military disease as well as for the direction of our efforts in the entire field of immunoprophylaxis.

Proposed Course of the Project: The study of Eaton agent in volunteers will be enlarged in the next year. Primary emphasis will be placed on the response of volunteers to Eaton agent grown as PPLO on artificial medium. An attempt will be made to select an attenuated variant of Eaton agent for immunoprophylaxis. If antibody development can be stimulated without the production of disease, the prospects for prevention of cold agglutinin positive pneumonia are good, since the Eaton volunteer study indicated that antibody protects against illness.

Experimental infection with adenoviruses by the enteric route will be pursued on an increasing scale during the next year. Type 4 adenovirus, which together with Type 7 is responsible for most of the acute respiratory disease associated with adenovirus in the military, will also be investigated for its capacity to infect by the lower intestinal route. The failure of enterically seeded Type 7 to infect the upper respiratory passages will be confirmed and infection contact experiments will be performed. If communicability of adenovirus 7 given by the enteric route can be shown to be minimal or non existent, then large scale immunication trials will be carried out in a prison or in the military.

Part B Included: Yes

Serial No. NIAID 72 B

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Chanock, R. M., Rifkind, D., Kravetz, H. M., Johnson, K. M.: Respiratory disease in volunteers infected with Eaton agent; a preliminary report. Proc. Nat. Acad. of Sci. 47: 887-890, 1961.

Kapikian, A. Z., Chanock, R. M., Reichelderfer, T. E., Ward, T. G., Huebner, R. J., Bell, J.A.: Inoculation of human volunteers with para influenza virus type 3. J.A.M.A. 178: 537-541, 1961.

Rifkind, D., Chanock, R. M., Kravetz, H. M., Johnson, K. M., Knight, V.: Ear involvement (myringitis) and primary atypical pneumonia following inoculation of volunteers with Eaton agent. Am. Rev. Respir. Dis. In press.

Chanock, R. M., Johnson, K. M.: Infectious Disease: Respiratory Virus. Annual Review of Med. 12, 1961.

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-76-A
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

A. Project Title: Ecologic studies of fungi pathogenic to man.

Principal Investigator: Chester W. Emmons

Other Investigators: H. F. Hasenclever, Willard Piggott, and Arthur Greenhall

Cooperating Units: Rabies Control Unit, Trinidad, W.I.

Man Years (calendar year 1961):

Total:	1 3/12
Professional:	6/12
Other:	9/12

Project Description:

Objectives:

To find new environmental habitats of fungi which cause systemic mycoses; to investigate the factors which limit growth of these fungi to special habitats; to investigate methods of eliminating pathogenic fungi from specific habitats and to apply the information so obtained to studies of the epidemiology of the systemic mycoses.

Methods Employed:

Samples of soil were collected from sites suspected of being sources of infection in individual cases of mycoses, from types of habitats known to harbor fungi in order to extend experience in this study and repeatedly in certain selected sites known to harbor pathogens in intensive studies of their ecology and associated saprophytic microflora. The specimens were processed by conventional methods of mouse inoculation with soil suspensions.

Specimens of soil were collected near houses infested by bats in Trinidad in cooperation with the Rabies Control Unit of Trinidad and in cities in eastern Maryland, Delaware and Virginia under trees used by starlings as roosting sites.

Part B included.

Major Findings:

Histoplasma capsulatum was isolated for the first time from a congested business district of a city where it was collected in a small park in Washington, D. C. This fungus was isolated also from soil samples taken in Trinidad from the soil adjacent to houses infested by bats. Studies of the association of Cryptococcus neoformans with pigeon excreta has been confirmed in many additional collections.

Significance to bio-medical research and the program of the Institute:

The isolation of Histoplasma from a congested urban area probably explains some of the urban Histoplasmin sensitivity which has been previously a puzzling epidemiologic problem. Histoplasma was isolated in Trinidad, extending our knowledge of the occurrence of the fungus in this area and its association with the house bat.

Proposed Course of Project:

The project will be continued indefinitely.

Part B Included: Yes

PHS - NIH Serial No. NIAID-76-A
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Emmons, C. W. Isolation of *Histoplasma capsulatum* from Soil in Washington, D. C. Public Health Repts. 76:591-596 , 1961.

Honors and Awards relating to this project:

Elected Councilor-at-Large, American Society of Microbiologists, July, 1960.

Appointed Board Member of the American Academy of Microbiology for a three-year term.

Chairman, Standards and Examinations Committee for Mycology, American Academy of Microbiology.

Member of Study Section on Mycology and Bacterial Diseases, NIH.

Annual Lecture of the Mycological Society of America at Purdue University, August, 1961.

Invitational Lecture, Japanese Mycological Society at Sendai, Japan.

Presentation of two papers entitled: "Pulmonary Cryptococcosis" and "The Fungi of Chromoblastomycosis" at the II Congreso Latinoamericano y I Nactional de Microbiologia, San Jose, Costa Rica, December, 1961.

Serve on Editorial Boards of: Mycologia, Journal of Bacteriology, Journal of Antibiotics and Chemotherapy, Mycopathologia.

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-76-B
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

B. Project Title: *In vivo* tests of antimycotic drugs and antibiotics.

Principal Investigator: Chester W. Emmons

Other Investigators: Benjamin Prescott, Willard Piggott, John P. Utz.

Cooperating Units: Medical Physiological Bacteriology Section and Laboratory of Clinical Investigations. (LID-64-A); LCI-23(C).

Man Years (calendar year 1961):

Total:	1 10/12
Professional:	3/12
Other:	1 7/12

Project Description:

Objectives:

To test the efficacy and safety of new drugs and antibiotics in experimental mycoses in mice and cooperate with the Laboratory of Clinical Investigations in clinical trials.

Methods Employed:

Mice are infected intravenously with a dose of fungus cells determined in prior experimental work to be sufficient to kill untreated (control) mice in 14-21 days. Treated mice are given test drugs at doses up to tolerance and effectiveness of drug is measured by its ability to extend survival time of treated mice and to clear the animals of infection as determined by autopsy and culture of the surviving animals when experiment is terminated. Collaborator has provided some of drugs tested and has synthesized drugs on his initiative or of types suggested by principal investigator.

Major Findings:

The effectiveness of the new antibiotic X5079C and its use in combination

with Amphotericin B have been further investigated. No synergistic activity could be observed. Methods of assay of X5079C in serum and urine have been developed and improved.

Significance to bio-medical research and the program of the Institute:

Presently available antimycotic drugs are too toxic and too ineffective for safe and ideal clinical use. Experimental therapy in animals should precede clinical trial.

Proposed Course of Project:

This project will be continued indefinitely.

Part B included. Yes

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-76-B
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Emmons, C. W. Chemotherapeutic and Toxic Activity of the Antifungal Agent X-5079C in Experimental Mycoses. The Am. Rev. Resp. Dis. 84: 507-513, Oct. 1961.

Emmons, C. W. and Piggott, W. R. Bioassay of the Antifungal Agent X-5079C in Serum of Patients. The Am. Rev. Resp. Dis. 84:534-537, Oct. 1961.

Utz, John P., Andriole, Vincent T. and Emmons, C. W. Chemotherapeutic Activity of X-5079C in Systemic Mycoses of Man. The Am. Rev. Resp. Dis. 84:514-528, Oct. 1961.

Honors and Awards relating to this project:

Appointed to WHO Expert Advisory Panel on Parasitic Diseases for a term of 5 years.

Appointed on Scientific Advisory Committee, Institute of Microbiology, Rutgers - The State University, New Brunswick, N. J. for a term of three years.

Member of Committee on National Index of Fungus Cultures, Quartermaster Research and Development, National Academy - Research Council, Natick, Massachusetts, for a term expiring June, 1963.

Special Lecturer on Medical Mycology, G. W. University, Fiscal Year beginning August 1960.

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-76-C
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

C. Project Title: Identification and study of new and unusual fungi from mycoses.

Principal Investigator: Chester W. Emmons

Other Investigators: Charles H. Bridges, Agricultural and Mechanical College, Texas.

Cooperating Units: Department of Agriculture, Agricultural College of Texas and many hospitals, diagnostic laboratories and individual physicians.

Man Years (calendar year 1961):

Total:	9/12
Professional:	3/12
Other:	6/12

Project Description:

Objectives:

To study new and poorly known mycoses and their etiologic agents; to test the virulence of unusual strains of pathogenic fungi and to investigate the geographic distribution of mycoses; to support and encourage medical mycology in laboratories in the United States and abroad where trained services in mycology are lacking or deficient.

Methods Employed:

Routine methods of fungus identification, adaptation of such methods when necessary, tests of pathogenicity and virulence in experimentally infected animals.

Major Findings:

A new mycosis causing nasal granulomas in horses caused by *Entomophthora coronata* has been described and a paper submitted for publication. Studies of fungi causing nasal granulomas in cattle are also under study.

Part B included.

Studies of Hyphomycosis and of unusual types of Mycetoma and chromoblastomycosis are under study.

Significance to bio-medical research and the program of the Institute:

The number, frequency and geographic distribution of mycoses are imperfectly known. This project has contributed significantly to elucidating some of the problems involved and demonstrating new mycoses.

Proposed Course of Project:

Project will be continued indefinitely.

Part B Included: Yes

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-76-C
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Bridges, Charles H. and Emmons, Chester W. A Phycomycosis of Horses Caused by *Hyphomyces destruens*. J.A.V.M.A. 138:579-589, June 1, 1961.

Emmons, Chester W. and Bridges, Charles H. *Entomophthora coronata*, the etiologic agent of a phycomycosis of horses. Mycologia. (In press).

Bridges, Charles H., Romane, William M. and Emmons, Chester W. A Phycomycosis of Horses caused by *Entomophthora coronata*. J. Amer. Vet. Med. Assoc. (In press).

Honors and Awards relating to this project:

None

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-78-A
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

A. Project Title: Biochemistry and Physiology of Pathogenic Fungi. (*In vitro* studies of the action of antifungal agent on pathogenic fungi).

Principal Investigator: George W. Lones

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 1 4/12
Professional: 8/12
Other: 8/12

Project Description:

Objectives:

To investigate the quantitative relationships of antifungal drugs with respect to fungicidal and fungistatic action; to study certain physical and chemical factors influencing this action; to study the effects of these drugs on certain metabolic activities of fungi; to study the emergence of strains resistant to these drugs; and ultimately to understand the mechanism of action of certain of the antifungal antibiotics.

Methods Employed:

Examination of the properties of the new antibiotic, X-5079C, has continued. Conventional methods are used for determining the quantitative relationships of growth of *Histoplasma capsulatum* and *Blastomyces dermatitidis* in the presence of varying concentrations of the antibiotic in suitable media under a variety of conditions. The effect of amino acids, growth factors, inorganic salts and other supplements on the activity of the antibiotic in a defined medium are studied. Convential methods are used for the study of the effect of the antibiotic on respiration and assimilation of *H. capsulatum* in the Warburg apparatus. Chemical methods are used to determine the effect of the antibiotic on synthesis of cellu-

Part B included.

lar substances in H. capsulatum.

Major Findings:

Several strains of Blastomyces dermatitidis tested in the yeast form have been found to be more sensitive to X-5079C than H. capsulatum. As in H. capsulatum the mycelial form is less sensitive than the yeast form. Respiration of H. capsulatum and assimilation of ammonia by this organism are not inhibited by antibiotic X-5079C. Synthesis of nucleic acids, particularly deoxynucleic acid, is inhibited by the antibiotic to a greater extent than synthesis of cell protein. To a certain degree, antibiotic X-5079C activity, in a defined medium can be neutralized by the addition of iron.

Significance to bio-medical research and the program of the Institute:

The need for new and more effective therapeutic agents for treatment of the systemic mycoses continues.

Antibiotic X-5079C has demonstrated impressive therapeutic effect in experimental mycoses in animals and encouraging results have been obtained in humans. The continued study of this and other antifungal agents is essential.

Proposed Course of Project:

It is planned to continue these studies. In view of the apparent effect of X-5079C on nucleic acid synthesis, the effect of nucleic acid-precursors on antibiotic activity is being examined. The mode of action of iron in reversing antibiotic activity warrants additional study. Attempts will be made to define more precisely the point of attack of antibiotic X-5079C on the metabolism of the fungus cell.

Part B Included: Yes

Serial No. NIAID-78-A
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Lones, G. W. and Peacock, Carl L. Effect of the Antifungal Agent X-5079C on the Growth of Histoplasma capsulatum in vitro. Am. Rev. Resp. Dis. 84:529-533, 1961.

Honors and Awards relating to this project:

None.

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-78-B
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

B. Project Title: Studies on the physiology of *Coccidioides immitis*.

Principal Investigator: George W. Lones

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 1 4/12
Professional: 4/12
Other: 1

Project Description:

Objectives:

The biochemistry and metabolism of this dimorphic pathogen have been little studied. It is the purpose of this project to obtain information on the metabolic characteristics of this microorganism, and in particular to discover metabolic differences in the two forms.

Methods Employed:

Shake culture techniques are employed to produce the parasitic and saprophytic morphologic modifications of *C. immitis* in sizeable quantities for examination by standard chemical and biochemical techniques.

Major Findings:

Two strains of *C. immitis* have been maintained in the spherule form. It has been found that both strains are capable of rapid growth in the mycelial form with mannitol as a carbon source. However, the spherules of these strains appear to be incapable of growth on mannitol, nor does mannitol appear to be oxidized by resting cells. This is the first absolute nutritional difference that has been found for the two forms of this fungus. Resting cells and cell-free extracts of mycelial form of the fungus have been prepared which are capable of oxidizing mannitol.

Significance to bio-medical research and the program of the Institute:

Progressive coccidioidomycosis is a disease with a high mortality. A better knowledge of the causative agent may favorably influence our diagnosis, prevention and treatment of the infection.

Proposed Course of Project:

Examination of the metabolism of the two morphological forms of the fungus will be extended. It is hoped that an improved method of disrupting the mycelial cells will enable the preparation of extracts of greater enzymatic activity. Comparison of extracts of mycelium and spherules will be made to attempt to define the enzymatic basis for the difference in behavior with respect to mannitol.

Part B not included.

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-79-A
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

A. Project Title: Immunity Studies with Pathogenic Fungi.

Principal Investigator: H. F. Hasenclever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 4/12
Professional: 1/12
Other: 3/12

Project Description:

Objectives:

To learn more about immunity to fungus diseases. Initially this investigation was conducted with coccidioidomycosis but has now been broadened to include diseases by other pathogenic fungi. Studies on cross immunity with various mycotic pathogens are in progress.

Methods Employed:

Mice are immunized by injecting either sublethal doses of viable fungi or with nonviable fungal preparations. Following the immunization period the immunized and control mice are challenged with viable homologous or heterologous fungi. The animals are observed daily and the deaths recorded.

Major Findings:

The results indicate that mice immunized with nonviable preparations of *Coccidioides immitis* spherules are more resistant to subsequent challenge with *C. immitis*. Mice given sublethal doses of *Candida albicans* cells are more resistant to *C. albicans* challenge than controls. Mice that have

Part B included.

received nonviable C. immitis preparation are somewhat more resistant than controls when challenged with C. albicans.

Significance to bio-medical research and the program of the Institute:

These studies have contributed to basic and fundamental information about required resistance to some of the pathogenic fungi.

Proposed Course of Project:

This project is to be continued.

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-79-A
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

None

Honors and Awards relating to this Project:

Presentation of a lecture on "Studies on Immunity to Coccidioidomycosis" at the Eleventh Annual Southwestern Conference on Diseases in Nature Transmissible to Man.

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-79-B
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

B. Project Title: Effect of Physiological alteration on the Pathogenesis of Yeast Infections.

Principal Investigator: H. F. Hasenclever

Other Investigators: Vincent T. Andriole

Cooperating Units: Laboratory of Clinical Investigations

Man Years: (calendar year 1961)

Total:	9/12
Professional:	5/12
Other:	4/12

Project Description:

Objectives:

To study the effect of alloxan diabetus, X-ray irradiation and cortisone on mice infected with various species of Candida or Torulopsis glabrata.

Methods Employed:

Lethality studies with Candida albicans, C. guilliermondii, C. parapsilosis and C. tropicalis have been completed using alloxan diabetic and X-ray irradiated mice. Growth rates in vivo of C. albicans and C. tropicalis have been determined in mice altered by these methods. The rate of growth of Torulopsis glabrata in vivo has been observed in mice with alloxan diabetus, X-ray irradiation and by cortisone treatment.

Major Findings:

These studies indicate that the susceptibility of mice to the species of Candida investigated is increased by alloxan diabetus and X-ray irradiation. The susceptibility of mice to T. glabrata is increased by alloxan diabetus, X-ray irradiation and cortisone treatment.

Part B included.

Significance to bio-medical research and the program of the Institute:

These studies have contributed to basic and fundamental information about resistance to pathogenic fungi.

Proposed Course of Project:

This project will be incorporated into another more extensive study pertaining to specific and nonspecific resistance to pathogenic fungi.

Part B Included: Yes

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-79-B
1. Infectious Diseases
2. Mycology Section
3. Bethesda, Maryland

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Andriole, V. T. and Hasenclever, H. F. Factors Influencing experimental fungus diseases. I. Alloxan diabetus. Bact. Proc. 1960.

Andriole, V. T. and Hasenclever, H. F. Alloxan diabetus and candidiasis in mice. J. Exper. Med. (submitted for publication).

Honors and Awards relating to this Project:

None

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-79-C
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

C. Project Title: Antigenic Studies on Candida.

Principal Investigator: H. F. Hasenclever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 4/12
Professional: 2/12
Other: 2/12

Project Description:

Objectives:

To learn more about the antigenic interrelationships of some of the provisional pathogens with particular emphasis upon the genus Candida. To determine the percentage of Candida albicans isolates from clinical materials that fall into antigenic Group A or Group B and to see if there is any correlation of these groups with isolation from an anatomical site.

Methods Employed:

Antisera to the desired species and strains of Candida are prepared in rabbits. Homologously and heterologously adsorbed antisera are utilized to show antigenic similarities or dissimilarities that exist within or between species. Tube agglutination reactions have been used predominantly. All C. albicans isolates from the clinical microbiology laboratory are being screened as to antigenic group. Collections of these isolations are to be made for 1 year. Thus far over 400 strains have been studied. The identity of all isolates is confirmed in this laboratory.

Major Findings:

These studies have shown that two antigenic groups are present in the species C. albicans. One of these groups is antigenically similar to C. tropicalis, while the other is identical to C. stellatoidea.

Part B included.

C. albicans Group A and Group B are identical with respect to morphology, biochemistry, and animal pathogenicity.

Significance to bio-medical research and the program of the Institute:

These studies have contributed basic information about some of the antigenic relations of the yeast-like fungi.

Proposed Course of Project:

To be continued but with emphasis upon the separation of soluble group A and B antigens and the clinical relationship of C. albicans Group A and B.

Part B Included: Yes

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-79-C
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Hasenclever, H. F. and Mitchell, William O. Antigenic Studies with Candida. I. Observation of Two Antigenic Groups in *Candida albicans*. J. Bacteriol. 82, 570, 1961.

Hasenclever, H. F., Mitchell, William O., and Lowe, J. Antigenic Studies with Candida. II. Antigenic relation of *Candida albicans* Group A and Group B to *Candida stellatoidea* and *Candida tropicalis*. J. Bacteriol. 82, 574, 1961.

Hasenclever, H. F. and Mitchell, William O. Antigenic Studies with Candida. III. Comparative pathogenicity of *Candida albicans* Group A, Group B and *Candida stellatoidea*. J. Bacteriol. 82, 578, 1961.

Hasenclever, H. F. and Mitchell, William O. Antigenic studies of *Candida albicans*. Bact. Proc. 1961.

Honors and Awards relating to this Project:

Presentation of a lecture entitled: "Pathogenicity of *Candida albicans*. Antigenic Groups A and B." Round table mechanisms of Fungal Pathogenicity, 1961, at the annual meeting of the American Society for Microbiology.

Presentation of a lecture entitled: "The Prevalence of Histoplasmosis in Eastern United States", at the Potomac Chapter, American College of Chest Physicians, annual meeting, October, 1961.

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-79-D
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

D. Antigenic Studies on Cryptococcus.

Principal Investigator: John Bennett

Other Investigators: H. F. Hasenclever

Cooperating Units: Laboratory of Clinical Investigations.

Man Years (calendar year 1961):

Total:	9/12
Professional:	7/12
Other:	2/12

Project Description:

Objectives:

To study the varied immunologic responses to naturally occurring systemic fungal diseases in man and experimentally induced infections in animals, and to apply these findings to the diagnosis and prognosis in patients.

Methods Employed:

Samples of serum and spinal fluid obtained before, during, and after specific treatment for various mycoses, particularly cryptococcosis, are being examined for:

A. antibodies, by means of
    1. complement fixation tests
    2. immunofluorescense
    3. blocking techniques
    4. agglutination
B. antigens by means of
    1. complement fixation
    2. precipitation

Part B not included.

Delayed hypersensitivity to antigenic fractions of C. neoformans is being studied in animals prior to patient trial. The clinical material for these studies was obtained from 25 patients with cryptococcosis, plus control specimens.

Major Findings:

An anticryptococcal rabbit serum of great potency has made possible the demonstration of cryptococcal polysaccharides in body fluids at concentrations down to 1-5 mcg.per ml. These polysaccharides seem to appear in spinal fluid and blood of patients with extensive disease.

Significance to bio-medical research and the program of the Institute:

The above tests should prove valuable in the prognosis and/or diagnosis of cryptococcal disease. They may add to our understanding of host response to deep mycoses.

Proposed Course of the Project:

This project will be continued.

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-79-E
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

E. Project Title: Studies on the Toxicity of *Candida albicans*.

Principal Investigator: H. F. Hasenclever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	9/12
Professional:	3/12
Other:	6/12

Project Description:

Objectives:

To study the toxic reactions of *Candida albicans* for mice and the acquisition of specific and nonspecific tolerance to these toxic substances.

Methods Employed:

The lethal manifestations of *C. albicans* are demonstrated when mice are injected intravenously with 10 million yeast cells. Death follows four to 12 hours after injection. *C. albicans* cells are cultured in glucose neopeptone broth for 48-72 hours and collected and washed before injection.

Major Findings:

Mice that have been infected with *C. albicans* intraperitoneally (a route of inoculation to which mice are quite resistant) 6 days before intravenous challenge survive 3-6 times as long as the control animals. Mice infected with a sublethal dose of *Coccidioides immitis*, or mice injected with nonviable *C. immitis*, or *Salmonella lipopolysaccharides* are tolerant to the toxicity of *C. albicans*. Sublethal infections with *C. stellatoidea* and to a lesser extent *C. guilliermondii*, *C. tropicalis*, *C. parapsilosis*, and *Torulopsis glabrata* also protect.

Infections with other species of pathogenic fungi protect in varying degrees. While tolerance or protection against the toxic manifestations of C. albicans can be observed, the mice still die of infection. Immune serum does not give protection. Large numbers of nonviable C. albicans cells give some protection.

Significance to bio-medical research and the program of the Institute:

These studies indicate the importance of nonspecific resistance to the toxic manifestations of candidiasis in mice and contribute to the knowledge of host-parasite relationships with pathogenic fungi.

Proposed Course of Project:

To be continued with emphasis upon nonspecific resistance to chronic fungus infections.

Part B not included.

PHS - NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID-79-F
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

F. Project Title: Role of the Reticulo-Endothelial System in Immunity against Pathogenic Fungi.

Principal Investigator: Donald F. Summers

Other Investigators: H. F. Hasenclever

Cooperating Units: None

Man Years: (calendar year 1961):
Total: 7/12
Professional: 6/12
Other: 1/12

Project Description:

Objectives:

To study the role of the reticulo-endothelial system in acquired resistance in laboratory animals against various pathogenic fungi.

Methods Employed:

Mice are injected intra-peritoneally with live Candida albicans cells or intra-venously with formalin-killed Candida. After several days the animals are sacrificed, and peritoneal macrophages are collected from normal and immunized mice. The macrophages are maintained during the experiments in monolayer flasks. The flasks are infected with Candida, and growth is followed microscopically and by plate counts for a twenty-four hour period.

Major Findings:

Early results suggest that "immune" macrophages suppress the growth of Candida to a greater extent than do normal macrophages.

Part B not included.

Significance to Bio-medical Research:

By utilizing the above *in vitro* techniques to study the reticulo-endothelial system, we hope to define the role played by this system in immunity against fungus infections.

Proposed Course of Project:

We intend to broaden the study to include other mycotic pathogens and reticulo-endothelial stimulants and depressants, i.e., endotoxins, steroids, etc. to gain knowledge concerning the contributions of specific and/or non-specific resistance to fungus infections.

LABORATORY OF BIOLOGY OF VIRUSES

Summary 1

80 - Mechanism of Oncogenic Activity of Tumor Viruses 5
80-A - Rabies Prophylaxis 8
81 - Genetics of Animal Viruses and Cells 11
82 - Mechanisms of Recovery from Viral Infection 13
83 - Genetics of Animal Viruses 16
84 - The Metabolism of Normal and Virus Infected Animal Cell Cultures 19
84-A - Effects of Fluorouracil Incorporation into Poliovirus 22
85 - Replication of Viruses 24
85-A - Relationship of Ribosomes to Viral Synthesis 28
86 - Biology of Mitochondria and its Relation to Endogenous and Viral Diseases 31
86-A - Characterization of Mitochondria 35
86-B - Investigations of Interference with Enzymatic Functioning of Mitochondria as a Mechanism for Carcinogenesis 39
86-C - Demonstration of Glucose Metabolism and Peptide Bond Formation by Isolated Brain and Liver Mitochondria 41
86-D - Comparison of the Properties of Crude and Crystallized Coxsackie A-10 Virus, of Cloudman S_{91}, Mouse Melanoma and of Mouse Muscle Nucleoprotein 43
87 - The Mechanism of Production of Poliovirus in Cultured Mammalian Cells 45
88 - Mechanism of Drug Resistance in Cultured Mammalian Cells 48
89 - Kinetics and Sites of Coxsackie Virus Multiplication 51
89-A - Virus Structure 54
89-B - Methods for Study of Nucleic Acid Structure 56

PHS-NIH
Summary Statement
Office of Chief
Laboratory of Biology of Viruses
Calendar Year 1961

Two major changes in the Laboratory of Biology of Viruses during 1961 have resulted in an expansion of its activities. Between July and September, all units of our original laboratory were moved from their scattered locations in Building 7 to be together on one floor in Building 5. Unfortunately, the move was to the third floor of Building 5 which had already been renovated to meet the specifications of the Laboratory of Immunology. Therefore, our program's activities had to be fitted into facilities planned for quite a different kind of research. This has made further renovations necessary, and to date none of these have been accomplished.

The second major change involved the transfer to LBV of the remainder of the Laboratory of Cell Biology after the retirement of Dr. Harry Eagle. With the exception of the loss of several positions and three laboratory modules, Dr. Eagle's whole laboratory was consolidated into LBV and continues to function in Building 10. This poses a problem of LBV operations being physically separated between two buildings and necessitates the continuation of two offices for the laboratory. The group in Building 10 has been organized into a Cell Biology Section with Dr. Norman Salzman as section chief responsible for scientific program. Dr. Leon Levintow, also a member of this Section, has been made assistant laboratory chief and is responsible for administrative operation of the laboratories in Building 10. Mr. Zadai and his staff give administrative service to both the Building 5 and Building 10 operations, but the person previously responsible for administration in Dr. Eagle's office continues in the same capacity for the Cell Biology Section and works through Mr. Zadai's office.

As the result of these changes there has been a reduction in the program in Building 10 from what it was under Dr. Eagle. One major project, Dr. Eagle's own cell metabolism studies, was completely eliminated. Two minor studies on poliovirus protein and in-born metabolic defects were curtailed due to loss of the professional personnel responsible for them, but an attempt is being made to have the studies continue by adding them on to the remaining intact programs. Three new young scientists have been added as temporary staff members in this Section. One, a biochemist from England, is here for one year as a Visiting Fellow, and the other two medical officers are here for two years military duty as a research associate and as a staff member. All three of these new people have been integrated into previously existing projects.

The program in Building 5 has been increased by the addition of two new projects and new personnel into already existing programs. Two visiting scientists, Drs. Takemori and Nomura, from the National Institute of Health in Tokyo, Japan, arrived in September and have been set up as a separate project, working in the field of virus genetics. Dr. Samuel Baron who had just completed a year's work in the laboratory of Dr. Isaacs at the National Institute for Medical Research in London, England, was added to our staff and is now responsible for a program on non-specific factors involved in recovery from virus infection with major emphasis on the substance, interferon. Dr. Arthur Malmon, a biophysicist, has been added to the staff of our Biophysical Unit, and Dr. Harvey Liebhaber, a research associate until July, was appointed as a permanent staff member to work with Dr. Takemoto on virus and cell inhibitors. A central tissue culture production unit has been established and is now supplying cell cultures to all the projects located in Building 5. A central tissue culture glassware washing unit has also been instituted.

Investigations of relationships between the virus and its host cell have continued to be the greatest effort of the laboratory. Four different units are working in this important general area, attempting to define the biochemical events, their sequence, site and inter-relationships. These studies represent both basic and practically oriented research. The basic and more direct goal is to determine the mechanism of protein and nucleic acid syntheses in the normal cell, the virus being used only as a self-replicating identifiable biochemical macromolecule. The other more remote goal is the development of knowledge concerning virus invasion and multiplication which might give a lead as to a logical point of attack in chemotherapy of virus diseases. The past year's work has been productive in this area. Studies on poliovirus in HeLa cells have shown that viral protein and RNA syntheses are closely coordinated in time and increase only a short time before whole infectious virus can be demonstrated. By the use of inhibitors there is evidence that the synthesis of viral RNA may be at least partially dependent upon protein synthesis. Viral protein antigens appear in both the nucleus and cytoplasm, but evidence is lacking as to whether this is the result of synthesis in both areas or migration after synthesis. Studies on vaccinia virus--a DNA virus--suggest that the synthesis of viral DNA and viral protein are dissociated. Work with Coxsackie virus--a RNA virus--shows precursor viral RNA present in the nucleus early after infection, declining by 3 hours and again increasing at 6 hours. Viral RNA in the cytoplasm decreases until 2 hours, then rises continuously to eventual high levels.

Three units have carried out studies on chemical and physical structure of virus as such. The protein coat of poliovirus has been isolated and found to contain no free N-terminal amino acid residues. The subunit of this protein appears to have a higher molecular weight than the predicted 20,000. A base analogue--5 fluorouracil--has been successfully

incorporated into the RNA of poliovirus to the extent of replacing 30% of the normal uracil, but with retention of biological properties of the virus. This "abnormal" virus continues to have all the properties of the parent normal virus as far as infectivity and host range susceptibility. Coxsackie virus has been shown by electron micrographs of purified virus to have an outer envelope and a dense inner core.

An important practical contribution to virus research technology has been made by the group studying virus inhibitors. The previously demonstrated inhibitor of plaque formation by certain genotypes of certain viruses has been identified as a sulfated polygalactose. Two different practical methods for its removal from agar have been developed as well as materials found which can neutralize the inhibitor. This has made it possible to obtain plaques with strains of ECHO viruses, myxoviruses, Arbor viruses, adenoviruses and rabies virus, all of which produced no plaques by standard methods. This approach has now been taken up by a number of basic virologists and a commercial company is interested in using methods developed here for producing inhibitor-free agar.

A study of the mechanism of oncogenesis by polyoma virus has developed evidence that when the virus transforms normal cells to tumor cells it changes the genome of the cell in such a way that a new "foreign" cell antigen appears. This "foreign" antigen is rejected by the immunologically competent adult animal and tumor development is suppressed, whereas the suckling animal being immunologically tolerant cannot reject the new antigen and tumors develop. This demonstrates two important phenomena: 1) the interaction of a virus with a mammalian cell can induce a genetically stable change in the antigenic components of the cell, and 2) immunological competence may be the limiting factor in the progressive development of a tumor after transformation has occurred.

Important contributions have been made in the general area of normal cell metabolism unrelated to virus infection. In the application of the finding made last year that tetracycline antibiotics localized specifically on mitochondria and can be demonstrated by fluorescent microscopy, it has been found that melanin granules in melanoma tumors act like mitochondria thus adding another bit of evidence for the mitochondrial nature of these granules. The development of a mutant line of HeLa cells resistant to the toxic effects of a glucose analogue has led to the demonstration that this drug resistance is due to the presence of an inhibitor of hexose phosphorylation. This inhibitor may be related to alkaline phosphatase since the latter is 8 to 10-fold higher in the resistant cells. Studies using a DNA inhibitor in HeLa cells have produced evidence that synthesis of protein and RNA in the nucleus is DNA-dependent, whereas their synthesis in the cytoplasm is not. In a continuation of studies on inborn metabolic defects, initiated by another investigator under Dr. Eagle, current studies of cell cultures from gout

patients show these cells to produce and release large quantities of purines compared to normal cells.

New areas of investigation initiated late in the year include the genetics of viruses and cells in tissue culture systems and the study of the mechanism of recovery from virus infection with special emphasis on interferon.

Serial No. NIAID-80
1. Biology of Viruses
2. Viral Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Mechanism of oncogenic activity of tumor viruses

Principal Investigator: Dr. Karl Habel

Other Investigators: Dr. Lowell Glasgow

Coperating Units: None

Man Years:

Total:	3 8/12
Professional:	1 9/12
Other:	1 11/12

Project Description:

Objectives:

To determine biochemical and biological events occurring when a tumor virus changes a normal cell to one with malignant properties.

Methods Employed:

Polyoma virus is inoculated into hamster and mouse embryo tissue cultures. The virus and cell growth characteristics are then followed and cells checked for oncogenic transformation by transplantation into irradiated adult isologous hosts.

The importance of immunological factors in determining development of a gross tumor in an animal after transformation has taken place has been investigated through the use of polyoma tumors established as transplantable lines. These are used for quantitative challenge of animals immunized with virus or tumor antigens.

Part B included: Yes

Major Findings:

We have not been able to confirm the rapid in vitro transformation of hamster tissue culture cells reported by others. However, one of four virus carrier cultures in mouse embryo cells has transformed after one year of in vitro maintenance.

Adult mice on inoculation with polyoma virus do not develop tumors in spite of virus multiplication, whereas the same inoculum in newborn mice produces tumors. Evidence suggests that this is due to the difference in immunological competence of the adult as compared to the newborn mouse. Through tolerance experiments, quantitative challenge with a polyoma transplantable tumor in irradiated adults, demonstration of immunity to challenge with the transplant in adults immunized with virus or with tumor, all suggest the presence of a "foreign" antigen in polyoma virus-induced tumor cells. Resistance of virus-immune adults to challenge with transplantable polyoma tumors is not related to antiviral antibodies but is cell-mediated like other types of transplantation immunity. This resistance is specific for polyoma-induced transplantable tumors but can be overcome by a large tumor challenge. It is postulated that polyoma virus on inoculation into both newborn and adult mice transforms normal cells into tumor cells and that these tumor cells contain an antigen "foreign" to the mouse which is recognized and rejected by the immunologically competent adult but tolerated by the newborn.

Significance to Bio-medical Research and the Program of the Institute:

The ability of the host to react immunologically to the "foreign" antigen present in the polyoma virus-transformed tumor cells appears to be the important factor determining whether or not a gross tumor will develop. This gives a logical explanation of why the occurrence of polyoma tumors is a rarity under natural conditions in spite of the fact that many mouse populations are heavily infected.

If the phenomenon of a virus-induced "foreign" antigen can be demonstrated in transformed cells of other tumor virus host systems and the immunological control of tumor development established as a common occurrence, it would go far in defining one major parameter in the problem of cancer control.

Proposed Course of the Project:

The "foreign" antigen in polyoma tumor cells will be investigated as to its nature and location. It will be sought for in cells transformed by polyoma virus in vitro. The phenomenon of foreign tumor antigen and immunological control of tumor development will be checked in other tumor virus systems, such as leukemias.

Serial No. NIAID-80

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Habel, Karl. Resistance of Polyoma Virus Immune Animals to Transplanted Polyoma Tumors. Proc. Soc. Exp. Biol. & Med. v106, 722-725, 1961.

Honors and Awards relating to this project:

Appointment of Dr. Habel as an Editor of VIROLOGY.

Serial No. NIAID-80A
1. Biology of Viruses
2. Viral Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Rabies Prophylaxis

Principal Investigator: Dr. Karl Habel

Other Investigators: None

Cooperating Units: Expert Committee on Rabies, World Health Organization

Man Years:
Total: 1 2/12
Professional: 3/12
Other: 11/12

Project Description:

Objectives:

To develop a practical, safe and refined vaccine for the prevention of rabies in man.

Methods Employed:

Adaptation of rabies virus to growth in a tissue culture system utilizing cells which would be a practical source for mass production of vaccine for use in humans.

Major Findings:

Fixed virus adapted to growth in chicken embryo tissue culture has been carried through 41 passages. Slow virus multiplication and relatively low yields have characterized all passages. Attempts have been made to determine factors which might be suppressing virus propagation. The combination of two characteristics of rabies virus seem to be responsible: thermal instability of the virus and the production of interferon in

Part B included: Yes

the cultures. It has not been possible thus far to eliminate these factors.

In the course of this work the development of cytopathic effect of the adapted virus on the chick-embryo cells has made available an in vitro system for the quantitation of anti-rabies neutralizing antibodies. Furthermore, by the use of this virus-cell system, it has been possible for the first time to obtain plaques with rabies virus.

Significance to Bio-medical Research and the Program of the Institute:

A tissue culture source of virus for the production of rabies vaccine for use in man would represent a great advance over the crude tissue suspensions used today and would probably eliminate the serious, even fatal reactions that occasionally occur due to the vaccine.

The development of an in vitro method for assaying antirabies neutralizing antibodies will greatly simplify serum surveys involved in ecology studies in animals as well as immunization studies in man.

Plaque methods when refined will make it possible to do basic investigations concerning the biochemical nature of the virus, and details of its intracellular multiplication.

Proposed Course of the Project:

Efforts to increase the yield of virus from tissue cultures will continue. Studies will be initiated to determine whether rabies is a DNA or RNA virus.

Serial No. NIAID-80A

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Atanasiu, P., D. A. Cannon, D. J. Dean, J. P. Fox, K. Habel, M. M. Kaplan, R. E. Kissling, H. Koprowski, P. Lepine, F. Perez Gallardo. Rabies Neutralizing Antibody Response to Different Schedules of Serum and Vaccine Inoculations in Non-Exposed Persons: Part 3. Bull. Wld. Hlth. Org. 25:103-114, 1961.

Honors and Awards relating to this project:

Reappointment of Dr. Habel for a third 5-year term as a member of the WHO Expert Committee on Rabies.

Serial No. NIAID-81
1. Biology of Viruses
2. Viral Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Genetics of Animal Viruses and Cells

Principal Investigator: Dr. N. Takemori

Other Investigators: Dr. S. Nomura

Cooperating Units: None

Man Years:

Total:	1
Professional:	8/12
Other:	4/12

Project Description:

Objectives:

To develop and study genetic mutant virus and cell strains and their interaction.

Methods Employed:

Mutant strains of polioviruses are sought under various selective conditions: for example, mutants capable of forming large and small plaques in the presence of certain inhibitors, heat resistant virus particles, strains requiring high cystine content of medium, etc. By altering the selective conditions, it is hoped that strains of virus will be developed which have various combinations of demonstrable genetically-stable characters. Once these are established then by simultaneously infecting cells in tissue culture with two different strains and testing all virus progeny for markers, various recombinations will give information concerning the loci of the genic materials responsible.

Part B included No

The work with cell lines involves the use of susceptibility to certain viruses as the genetic markers to be studied. Cell lines resistant to certain viruses are developed from clones of an originally susceptible cell. The nature of the resistance will be investigated and the resistant cells used for segregating virus mutants that may be able to overcome their resistance.

Major Findings:

This project was just initiated on September 1, 1961, with the arrival of Drs. Takemori and Nomura as Visiting Scientists from the National Institute of Health in Tokyo. They have brought with them a number of mutant strains of polioviruses and certain resistant human cell lines to be used in these studies.

Significance to Bio-medical Research and the Program of the Institute:

Relatively little work has been done in the field of animal virus genetics and yet the sensitivity of viral technics and the specificity of the biochemical units involved make the animal viruses probably the best material for definitive work on genetic events occurring in animal cells. A further parameter is added by using susceptibility or resistance to virus infection as a means of studying some aspects of the genetic potentialities of the cells as such.

Proposed Course of the Project:

It is planned that this project will continue for the next two years during the remainder of the Visiting Scientists' appointments of Drs. Takemori and Nomura.

Serial No. NIAID-82
1. Biology of Viruses
2. Viral Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Mechanisms of Recovery from Viral Infection

Principal Investigator: Dr. Samuel Baron

Other Investigators: Charles E. Buckler

Cooperating Units: Dr. Alick Isaacs, National Institute for Medical Research, London, England.
Dr. R. M. Friedman, NCI.

Man Years:

Total:	8/12
Professional:	4/12
Other:	4/12

Project Description:

Objectives:

Studies of the factors which are responsible for recovery from viral infection.

Methods Employed:

Guinea pigs, whose immune mechanisms have been suppressed by X-irradiation or metabolic antagonists are infected with vaccinia virus and studied for growth of virus, elimination of virus, antibody production, delayed hypersensitivity and interferon production.

Embryonated eggs, which are too young to have an immunologic response, are infected with virus and are studied for mortality, viral growth, interferon production, interferon sensitivity, and ability of explanted tissues to support virus growth and to eclipse virus.

Part B included: Yes

Cell cultures, under varying degrees of oxygenation, are studied for growth of virus and sensitivity to interferon by the plaque assay method. Mice, infected with influenza virus and maintained under 50% oxygen, are observed for mortality due to influenzal pneumonia.

Major Findings:

Guinea pigs whose antibody and delayed hypersensitivity responses were blocked, recovered from vaccinia virus infection as rapidly as did normal guinea pigs.

Embryonated eggs and mouse embryos were found to lack the interferon mechanism during the first third of embryonic development and to rapidly develop interferon responsiveness thereafter. The ability of chick embryos to survive virus infection was observed to closely parallel the development of the interferon mechanism and this recovery occurred in the absence of an immune response.

Diminished oxygen tension on cell cultures was observed to prevent the multiplication of viruses in these cells. Viruses most sensitive to interferon were most inhibited by lack of oxygen. Low oxygen tension increased interferon activity and high tensions decreased interferon activity. High oxygen tensions applied to mice with influenza pneumonia, resulted in increased mortality.

Significance to Bio-medical Research and to the Program of the Institute:

These findings suggest that the immune response is not essential for recovery from the primary viral infection and that interferon and the inflamatory response (low pH, low oxygen tension, etc.) may be more important in the recovery process. Understanding of the factors which govern the recovery process may allow study of methods to enhance recovery from viral infection.

Proposed Course of the Project:

These studies will continue with attempts to determine: 1) the mechanism by which virus is destroyed during recovery, 2) the role of interferon and the inflamatory response in recovery from reinfection, 3) the effect of the absence of interferon on the recovery process, and 4) attempts to hasten recovery by stimulating the recovery mechanism.

Serial No. NIAID-82

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Isaacs, Alick and Baron, Samuel. Antiviral action of interferon in embryonic cells. The Lancet, Vol. 2, 946, Oct. 29, 1960.

Baron, Samuel and Isaacs, Alick. Mechanism of recovery from viral infection in the chick embryo. Nature, Vol. 191, No. 4783, 97, July 1, 1961.

Baron, Samuel, Porterfield, J. S., and Isaacs, Alick. The influence of oxygenation on virus growth. I. Effect on plaque formation by different viruses. Virology, Vol. 14, No. 4, 444, Aug. 1961.

Isaacs, Alick, Porterfield, J. S., and Baron, Samuel. The influence of oxygenation on virus growth. II. Effect on the antiviral action of interferon. Virology, Vol. 14, No. 4, 450, Aug. 1961.

Friedman, Robert M. and Baron, Samuel. The role of antibody in recovery from infection with vaccinia virus. J. of Immunology, Vol. 87, No. 4, 379, Oct. 1961.

Friedman, Robert M., Buckler, Charles E., and Baron, Samuel. The effect of aminomethylpteroylglutamic acid on the development of skin hypersensitivity and on antibody formation in guinea pigs. J. Exp. Med., Vol. 114, No. 2, 173, Aug. 1961.

Baron, Samuel and Isaacs, Alick. Interferon and natural recovery from virus diseases. New Scientist, Vol. 11, 81, 1961.

Honors and Awards relating to this project:

None

Serial No. NIAID-83
1. Biology of Viruses
2. Viral Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Genetics of Animal Viruses

Principal Investigators: Dr. K. K. Takemoto and Dr. H. Liebhaber

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	4 3/12
Professional:	2
Other:	2 3/12

Project Description:

Objectives:

1. To isolate, characterize, and investigate the chemistry and biology of virus mutants.

2. To develop methods for concentration and purification of viral mutants in order to obtain sufficient quantities of virus for chemical as well as biological investigations.

Methods Employed:

Genetically pure lines of virus are developed and characterized by plaque techniques. Mutants which form plaques differing from the parental type are isolated under various conditions and studied for genetic markers. The basis for the genetic differences is sought by biological and biochemical studies.

Part B included: Yes

Major Findings:

1. The basis for plaque size differences with encephalomyocarditis (EMC) virus has been found to be due to the presence of a sulfated polysaccharide in agar. This polyanion is released from the agar in soluble and highly active form during the autoclaving process. When used in the overlay medium, its inhibitory effect results in the restriction of plaque development.

2. Because of the extensive use of agar in current virological investigations, two useful procedures for preparing agar free of the sulfated polysaccharide have been developed. A simple method has been the addition to melted agar of a polycation, either DEAE-dextran or protamine, which forms an insoluble complex with the polyanion, rendering it ineffective. The other method involves chemical fractionation of agar resulting in a final product which is neutral and free of the sulfated polysaccharide.

3. Present investigations have indicated that a large number of viruses are affected by the agar polysaccharide. Plaque formation by enteroviruses (ECHO, polio, and Coxsackie), Arbor viruses (dengue), myxoviruses (influenza), and rabies virus is influenced by the sulfated polysaccharide. Plaque formation has now been demonstrated for these viruses by the use of inhibitor-free agar.

Significance to Bio-medical Research and the Program of the Institute:

Although the effect of polysaccharides on animal viruses has been recognized for a number of years, such studies have heretofore been limited primarily to myxoviruses. It is now evident that other animal viruses are influenced by polysaccharides. Viruses which have not produced plaques under agar can now be studied by highly quantitative plaque techniques using inhibitor-free agar.

Information concerning the mode of action of various virus and cell inhibitors should give an insight and possible new avenues of investigation into the mechanisms of viral replication.

Proposed Course of the Project:

1. Studies are currently in progress to determine the mechanism by which certain viruses are inhibited by the sulfated polysaccharides. The effects of these compounds on viruses and cells will be investigated.

2. Investigations will be continued on methods for demonstrating genetic interaction with animal viruses.

3. Various methods for large scale purification and concentration of viral mutants are being explored in order to obtain virus in sufficient quantities for studies on viral nucleic acid and protein.

Serial No. NIAID-93

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Takemoto, K. K. and Liebhaber, H. Virus-Polysaccharide Interactions. I. An Agar Polysaccharide Determining Plaque Morphology of EMC Virus. Virology 14, 456-462, 1961.

Liebhaber, H. and Takemoto, K. K. Alteration of Plaque Morphology of EMC Virus with Polycations. Virology 14, 502-504, 1961.

Honors and Awards relating to this project:

None

NIAID 84
Laboratory of Biology of Viruses
Section on Cell Biology
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: The Metabolism of Normal and Virus Infected Animal Cell Cultures

Principal Investigator: Dr. Norman P. Salzman

Other Investigators: Dr. Aaron J. Shatkin, Dr. Donald N. Planterose, Dr. Chiaki Nishimura and Mr. Edwin D. Sebring

Cooperating Units: None

Man Years (calendar year 1961)
Total: 6-3/12
Professional: 2-3/12
Other: 4

Project Description:

Objectives: To study the interaction of vaccinia virus and cultured animal cells. To analyze the series of events which initiate the infective process, and to study the mechanisms involved in the synthesis of the viral nucleic acid and viral proteins.

To study control mechanism and pathways of nucleic acid synthesis in uninfected cell cultures.

Major Findings:

1. Purification of Vaccinia Virus. The purification of vaccinia virus from infected HeLa cell cultures has been studied. By differential centrifugation, extraction with halogenated solvents and density gradient centrifugation, material has been obtained which appears to contain only vaccinia virus when examined by electron microscopy. However, only small quantities of material are obtained and methods to obtain larger quantities of virus are being studied.

2. Formation of Viral Components in Vaccinia Infected Cell Cultures. It was previously reported that the synthesis of viral DNA preceded by several hours the time of formation of infectious virus and of several species of viral protein. The synthesis of DNA can be blocked at any time by the

Part B included

addition of the inhibitor, 5-fluorodeoxyuridine. Preliminary studies would indicate that under conditions where viral DNA synthesis is blocked, the synthesis of viral protein continues, and that the synthesis of viral DNA and viral protein can be dissociated. A system that would give the viral proteins in soluble form would be of great value in studying the time course of their formation.

3. Control Mechanisms in HeLa Cell Cultures. We previously reported that there was an apparent linkage in the synthesis of nuclear RNA and protein and DNA, and that such a linkage did not exist for cytoplasmic RNA and protein. We have tried to establish if this represents a mechanism by which levels of various enzymes in the cell would vary in response to the rate of synthesis of DNA. When nuclear RNA and protein synthesis are blocked, and a 100 per cent increase in cytoplasmic protein occurs, there is a parallel increase in two cytoplasmic enzymes which have been measured. Thus the protein formed under these conditions is functional.

Procedures for the assay of nuclear enzymes have been devised, and cell fractionation studies have been studied.

4. Synthesis of Nucleic Acids in Euploid Cultures Derived from Patients with Gout. Skin biopsies were obtained from gout patients who had been studied and found to be overproducers of purines. Rapidly growing euploid cultures have been established from these biopsies. They are characterized by an unusually high content of DNA per cell which may be indicative that they have become polyploid. Their chromosome number is being studied. Large quantities of purines are produced by "gout" cultures and this is excreted into the medium. This is in contrast to aneuploid cultures. The behavior of normal "euploid cultures" is presently being studied.

Significance to the Program of the Institute: The effect of viruses on animal cells, the manner in which viruses replicate, and the effect of inhibitors on these processes is of relevance to an understanding of viral infection, and may conceivably suggest new areas of exploration in relation to viral chemotherapy.

Proposed Course of Project:

1. Methods to obtain larger quantities of pure virus will be investigated. The studies on the mechanism of virus synthesis will be carried out using antisera to the various virus proteins. The mechanism of cytoplasmic DNA synthesis that occurs in vaccinia infection will be analyzed.

2. Levels of nuclear and cytoplasmic enzymes will be measured and related to DNA synthesis.

3. Differences between normal and gout euploid cultures will be examined to determine the nature of the lesion in cultures of cells for gout patients.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Salzman, N.P. and Sebring, E.D. The Source of Poliovirus Ribonucleic Acid. Virology 13: 258-260, 1961.

Salzman, N.P. Animal Cell Cultures. Science 133: 1559-1565, 1961.

Salzman, N.P. Interrelationships in the Synthesis of RNA, DNA and Protein in Normal and Virus Infected Cell Cultures. In press in University of Texas publication "The Molecular Basis of Neoplasia," 1961.

Salzman, N.P. DNA, Virus and Protein Synthesis in Animal Cell Cultures. In press, Proc. Tissue Culture Association.

Honors and Awards relating to this project:

Presented talks at the following:

February 23-25, 1961	- Symposium on "The Molecular Basis of Neoplasia." Sponsored by the M.D. Anderson Hospital.
February 27, 1961	- Department of Bacteriology, University of Texas, Austin, Texas.
March 2, 1961	- The Institute of Microbiology, Rutgers University.
March 16, 1961	- Department of Microbiology, The University of Chicago.
June 6-8, 1961	- Syverton Memorial symposium sponsored by the Tissue Culture Association.

NIAID 84-A
Laboratory of Biology of Viruses
Section on Cell Biology
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Effects of Fluorouracil Incorporation into Poliovirus

Principal Investigator: Dr. William H. Munyon

Other Investigators: Dr. Norman P. Salzman

Cooperating Units: None

Man Years (calendar year 1961)
Total: 2-3/12
Professional: 1-3/12
Other: 1

Project Description:

Objectives: To determine the chemical and genetic effects of the incorporation of the base analogue, 5-fluorouracil, into the nucleic acid of poliovirus.

Methods Employed: Poliovirus replication has been carried out in the presence of the nucleic acid analogue, 5-fluorouracil (5FU). The resulting virus has been purified and its properties studied.

Major Findings: 5FU inhibits the synthesis of poliovirus at 10^{-3} and 10^{-2} M concentrations, but synthesis is normal at 10^{-4} M. By chemical fractionation of the virus RNA and by incorporation studies with 5FU-2-C^{14} it has been shown that the analogue is incorporated into the viral RNA as the nucleotide, and that the degree of incorporation is a function of the concentration of 5FU present during virus replication. At 10^{-4} M 5FU, 10% of the uracils in the virus are replaced by 5FU and at 10^{-3} M there is a 30% replacement of uracil by fluorouracil. The presence of 5FU in the viral nucleic acid does not alter its specific infectivity or its ability to produce plaques on a variety of subclones of susceptible cells, indicating a similar host range specificity.

Significance of Program to Institute: Since the genetic information of RNA viruses is contained in the nucleic acid component, the effect of the incorporation of base analogs, such as 5FU on the properties of the virus will give direct information regarding how viral RNA controls the synthesis

Part B not included

of the viral proteins. Also alterations in viral properties, such as antigenic structure and mutation rate, may suggest methods for viral chemotherapy.

Projected Course of Project: At present the protein of FU substituted poliovirus is being examined by the following methods; amino acid analysis, kinetics of neutralization of virus by specific antiserum and by the rate of heat inactivation. Alterations in genetic composition of FU substituted virus will be made by determining the rate of mutation to guanidine resistance of 5FU substituted and control virus.

Serial No. NIAID-85
1. Biology of Viruses
2. Biochemistry & Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Replication of Viruses

Principal Investigator: Dr. Hilton B. Levy

Other Investigators: Dr. Frank DeFilippes

Cooperating Units: None

Man Years:
Total: 3 2/12
Professional: 1
Other: 2 2/12

Project Description:

Objectives:

Broadly speaking, the objectives are to study the inter-relationships of nucleic acid metabolism and protein synthesis, with attention to examination of the role of the subcellular elements, and with emphasis on viruses as the nucleoprotein being studied.

The previous years' activities had produced evidence suggesting that the cell nucleolus was the site of production of specific RNA templates that were transferred to the cytoplasm for participation in the synthesis of a poliovirus protein, and that shortly thereafter another virus component was synthesized in the cell nucleus. The objectives for the past year were: 1) to gain information about the form in which RNA was transferred from the nucleolus to the cytoplasm; 2) to obtain independent evidence for the presence in poliovirus of two separate antigens, and 3) to achieve the synthesis of these components in cell-free systems.

Part B included: Yes

Methods Employed:

Details of methods are given under results, but briefly the procedures for the three above-mentioned objectives were in order as follows: 1) exposure of cells to radioactive nucleic acid precursors for a brief period of time, removal of the radioactive precursor from the medium, further incubation of the cells, and determination of the time course of translocation of the nucleic acid by means of high resolution autoradiography and cell fractionation techniques; 2) preparation of large quantities of virus, preparation therefrom of the protein, and analysis by immunoelectrophoresis, and 3) incubation of cell fractions with suitable radioactive protein precursors, and energy generating systems in order to synthesize protein in a cell-free system. The detection of specific virus protein was determined by immunological methods.

Major Findings:

1. Radioautography revealed that cells, exposed to tritiated cytidine for a short time incorporate the cytidine mostly into nucleoli, and practically not at all into the cytoplasm. Within a short time the radioactivity of the nucleolus goes down and that of the cytoplasma goes up. Cytoplasmic ribosomes, a major protein synthesizing system of the cell, isolated shortly after this short exposure of the cell to tritiated cytidine are virtually free of radioactivity, but as the nucleolus loses its radioactive RNA the ribosomal RNA gains in radioactivity. The simplest explanation for these observations is that ribosomal RNA, presumably specific for the synthesis of specific protein is made in the nucleolus, and is then transferred to the cytoplasm. Electron micrographs and ultra-microspectrophotometric analysis of cells, published in the literature, suggest that this transferred RNA is in the form of ribonucleoprotein particles. However, other evidence that we have raises the possibility that the nucleolar RNA may possibly break down to acid soluble materials before it is incorporated into ribosomes. Different methods from the ones we have been using will have to be employed to examine this possibility.

2. Only a beginning has been made on the second goal. We have been investigating a new polyacrylamide gel of presumed high resolving power, for use in immunoelectrophoresis. We have also begun to prepare the large amounts of virus needed for this work.

3. The procedures used to study the cell-free synthesis of poliovirus protein consisted briefly of infecting HeLa cells with the virus and after suitable short times, of preparing a microsomal cell sap and a nuclear fraction from them. Such subcellular entities were incubated with radioactive amino acids, suitable cofactors and an energy generating system, under conditions conducive to protein synthesis. Any virus protein synthesized would be expected to contain radioactive amino acids. Enough

carrier, non-radioactive pure virus was added to the system to give a precipitate with immune serum, and the radioactivity in such a precipitate compared to one made in a similar way with uninfected cells as starting material. The specific precipitate obtained using cytoplasmic fractions from infected cells had more radioactivity than that from controls, indicating that cell-free synthesis of virus protein had occurred, but the levels of incorporation have been too low to be totally convincing. With nuclei, the levels were also very low. Recently, we have succeeded in preparing more active cell fractions.

Significance to Bio-medical Research and the Program of the Institute:

This program is attempting to gain information about the details of virus reproduction in the cell. In so doing, knowledge about the functioning of the several parts of the normal cell should be developed. The emphasis has been, and will continue to be, on the relationship of the biochemistry to the biology of the problem.

Proposed Course of the Project:

For the forthcoming year it is hoped to be able to determine more definitely the relationship between nucleolar function and ribosome manufacture. The work on fractionation of poliovirus protein will continue, as will the attempts to achieve cell-free synthesis of the two virus components.

Serial No. NIAID-85

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Levy, Hilton B. Intracellular Sites of Poliovirus Replication. Virology, October 1961.

Levy, Hilton B. Metabolic Effects of Animal Viruses. Invited paper at a symposium of Society of General Physiology at Woods Hole, September 1961, and to be published as a chapter in a book on Respiration and Glycolysis. A 4-page summary is to be published in the January 1962 number of Journal of Physiology.

Barons, J. P., Nason, R. M., Friedman, C.M.O., Levy, H. B. and Barnett, E. V. Production of Antibody by Hypogammaglobulinemic Patients. J. Immunology, in press.

Honors and Awards relating to this project:

See publication #2. Dr. Levy was invited to be Chairman of a section on virus biochemistry at the Vth International Biochemistry Congress in Moscow.

Serial No. NIAID-85A
1. Biology of Viruses
2. Biochemistry & Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Relationship of Ribosomes to Viral Synthesis

Principal Investigator: Dr. Frank M. DeFilippes

Other Investigators: Dr. Hilton B. Levy

Cooperating Units: None

Man Years:

Total:	1	11/12
Professional:	1	
Other:		11/12

Project Description:

Objectives:

To investigate the synthesis of an animal virus in a tissue culture system by studying RNA derived from different cell fractions, and in particular, that derived from ribosomes which are the particles involved in soluble protein synthesis.

Methods Employed:

HeLa cells infected with poliovirus are grown in a radioactive medium. Cells are collected at different times and cell fractions are isolated. The ribose nucleic acid (RNA) is extracted and purified from these fractions with particular attention being given to the ribosomal RNA. The RNA is then degraded to the nucleotide level and the four nucleotides which comprise the RNA are separated on an ion exchange column. The individual nucleotides are concentrated and the total radioactivity of each nucleotide is compared to the other nucleotides. The specific activity of each nucleotide is also measured. Results with uninfected control cells are compared with those found for the nucleotides from infected cells.

Part B included: No

Major Findings:

When radioactive phosphorus (P^{32}) is administered to uninfected HeLa cells growing in suspension culture for a period of one hour, it is found that the greatest concentration of radioactivity (specific activity) is in the smaller size RNA which is generally called soluble RNA. The specific activity of ribosomal RNA is about one-half that of soluble RNA and about equal to that found for the remaining RNA which is neither soluble nor ribosomal.

When P^{32} is given to virus infected cells two hours after infection and the cells are harvested and fractionated at three hours, measurement of ribosomal radioactivity indicates that the new ribosomal RNA made between two and three hours is not significantly different from similar RNA isolated from control cells. The major difference seems to be a decrease in the specific activity of uridylic acid.

When the same experiment is performed between four and one-half and six and one-half hours after infection, the newly synthesized ribosomal RNA differs markedly in that its cytidylic and guanylic acid content is decreased and the specific activity of its pyrimidine nucleotides has changed with respect to similar RNA from uninfected cells. The base composition of the ribosomal RNA as measured by total phosphorus has not however, changed markedly in infected cells compared to control cells. The percent base composition of ribosomal RNA in HeLa cells is cytidylic acid 32.9; adenylic acid 17.3; uridylic acid 15.7, and guanylic acid 34.1.

Significance to Bio-medical Research and the Program of the Institute:

The program is designed to lead to a general picture of the synthesis of an animal virus by following events at a molecular level. It is hoped that the key steps involved in the conversion of the cellular machinery from normal metabolic activity to a virus producing system will be elucidated. Interference with these key steps may lead to new and general methods of arresting viral disease.

Proposed Course of the Project:

The total RNA of the cell will be extracted and an attempt will be made to separate the RNA of the particular parts of the cell directly from the total extract. A method has already been devised to separate the cytoplasmic and nuclear RNA of HeLa cells and I will attempt to fractionate these RNA materials even further. Resolution of nuclear RNA into chromatin and nucleolar material will be attempted using ion exchange resins, molecular sieves, sucrose and cesium chloride density gradients. The base composition of RNA newly synthesized after infection of HeLa cells with polio virus and possibly Krebs ascites cells with EMC virus should give a very general picture as to where viral precursor RNA is formed.

Viral protein antigen experiments are also anticipated. Here ultrasound will be used to fracture purified virus into its constitutent parts and an attempt will be made to develop antibody to these fragments, and also to see if the fragments can be used in antibody blocking experiments with a purified virus-antibody system. If successful these techniques might permit the identification of viral protein subunits made at an early time after infection. The techniques of immuno-electrophoresis will be used to examine the various cell fractions for these viral protein subunits.

Experiments designed to follow the intracellular fate of poliovirus with radioactive RNA, similar to those performed by Joklik and Darnell are planned. The major difference in approach will be a concentration of attention on intracellular RNA. Instead of disrupting virus infected cells with a sonic oscillator, gentle means of disrupting the cells will be used in order to try to find the existence of a long lived polymeric piece of virus RNA which might be a template for continued virus production. An attempt to find a piece of virus RNA with a sedimentation coefficient of at least 4S (MW r 25,000), similar to ribosomal precursor RNA will be made.

Also an investigation is planned to determine whether or not a correlation can be made with the time of synthesis of viral RNA and its base composition. If different parts of a single virus RNA molecule are made at different times and if these parts have a different base composition, an experiment where P^{32} is administered at two different times during the time course of production of viral RNA followed by a base analysis of these RNA's should reveal these differences.

Serial No. NIAID-86
1. Biology of Viruses
2. Biochemistry & Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Biology of mitochondria and its relation to endogenous and viral diseases

Principal Investigator: Dr. H. G. duBuy

Other Investigators: J. L. Showacre

Cooperating Units: Analytical Services Unit, Mr. H. G. McCann
Laboratory of Technical Development, NHI
Dr. Joseph Hayes

Man Years:
Total: 3 2/12
Professional: 1 2/12
Other: 2

Project Description:

Objectives:

To define the biological characteristics of normal mitochondria, and to investigate the relationship between mitochondria and virus replication.

Methods Employed:

The methods encompass the application of the cytochemical findings reported under project No. NIAID-86A. They also include Warburg metabolic techniques, determination of oxidative phosphorylation, tetracycline uptake, nucleoprotein content, and enzyme content determination by chemical and electrophoretic means.

The results are applied to in vitro cultivation of isolated mitochondria and the cultivation of viruses on such cultures.

Part B included: Yes

Major Findings:

A paper, containing the major findings, was presented at the 5th International Pigment Cell Conference, published in the Annals of the New York Academy of Sciences, 1961. Five series of experiments were presented in support of the theory that melanin granules represent modified mitochondria. One set of experiments showed that melanin granules selectively take up tetracycline, as do mitochondria of normal tissues. This latter evidence was given in an article published in Science, January 1961. It establishes for the first time convincing evidence for the precise localization of an antibiotic on particular subcellular elements, the mitochondria. It further supplies a tool to characterize mitochondria in living cells. It has supplied a great number of leads for the study of the role of mitochondria, in vivo, in various organs. These leads will be followed when opportunities are available.

A method for the quantitative determination of ATP (adenosine triphosphate) in crude extracts was developed, using the frozen Rabbit Psoas muscle as indicator. The details will be presented in a separate report. The availability of such a method is important, since in vitro systems will not synthesize in the absence of high energy phosphate.

With this method, it was established that acetone powders of brain mitochondria will phosphorylate ADP. It is planned to use such powders to enforce the phosphorylation of intact isolated mitochondria.

It was further found that acetone powders of brain mitochondria, besides glycolyzing, also oxidatively metabolized glucose. The precise pathway is under further investigation.

Significance to Bio-medical Research and the Program of the Institute:

The cultivation of mitochondria in vitro when accomplished should allow the cultivation in vitro of those viruses which multiply in the cellular cytoplasm. The selective uptake of tetracycline by mitochondria is useful in two major ways: 1) its antibiotic effect can be studied in respect to a specific subcellular element. For instance, the affinity of mitochondria of the host cell, and those of tetracycline-susceptible or tetracycline-resistant pathogens can be compared, and 2) tetracycline as an indicator of mitochondrial elements can be used to show the extent of mitochondrial breakdown, either as a result of techniques and media used to prepare subcellular fractions, or as a result of the incorporation of bacteria or viruses in certain intact host cells.

Proposed Course of the Project:

Mitochondrial fractions will be prepared by various extraction techniques, and in various media. Their intactness will be determined by microscopic observation, by the quantitative determination of tetracycline retained by the mitochondria, and by the biochemical determination of the number of complete enzyme systems, retained by the mitochondria after manipulation, in order to establish the optimal conditions for *in vitro* cultivation of mitochondria.

Serial No. NIAID-86

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

du Buy, H. G. and Showacre, J. L. Selective Localization of Tetracycline in Mitochondria of Living Cells. Science, Vol. 133, 196-197, January 20, 1961.

du Buy, H. G. and Woods, M. W. Segregation of cytoplasmic particles in green plants. Recent Advances in Botany II, 1489-1494, Univ. of Toronto Press.

du Buy, H. G., Showacre, J. L. and Hesselbach, M. L. Enzymic and other Similarities of Melanoma Granules and Mitochondria. Ann. N. Y. Acad. Sci., Dec. 1961.

Honors and Awards relating to this project:

None

Serial No. NIAID-86A
1. Biology of Viruses
2. Biochemistry & Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Characterization of mitochondria

Principal Investigator: Jane L. Showacre

Other Investigators: Dr. H. G. du Buy

Cooperating Units: None

Man Years:

Total	1 6/12
Professional:	8/12
Other:	10/12

Project Description:

Objectives:

To study the morphological and metabolic effects of viral and bacterial pathogens on infected cells.

Methods Employed:

Living cells were examined by light, phase and fluorescence microscopy following exposure to infectious agents (viruses, bacteria), fluorescent compounds (tetracycline), and other vital stains. Sources of cells were: primary and established tissue cell lines, normal and neoplastic tissues from animal organs and cultures of microorganisms (S. typhosa). Tissue culture cells were grown on coverslips and mounted either on regular slides for fluorescent studies, or in a special chamber for continued observation of living cells. Still and cine-photomicrographic records were made.

Part B included: Yes

Major Findings:

Cytopathogenic effect on single cells in tissue culture. A range of concentrations of tetracycline has been studied in tissue culture cells under conditions where decreased cell division and morphologic changes are minimized. This was preliminary to a study of the effects of the mitochondrially adsorbed tetracycline on virus multiplication.

Since primary monkey kidney cell cultures are well suited for cytologic study, and since several simian agents produce pronounced cytopathogenic effects, these agents are being used in conjunction with tetracycline in an effort to detect earlier CPE and relate its development to changes in normal cell constituents. Although no gross differences in intensity of staining between viral infected and non-infected cells have been noted, staining properties of inclusion bodies are being followed further.

Microorganisms. Observations on the fluorescence of extracellular S. Typhosa to which tetracycline was added showed two stages in the uptake of tetracycline. In the first, the drug appeared predominantly in the bacterial wall with small amounts in the cytoplasm, and in the second stage predominantly within the bacterial cytoplasm. Certain factors such as media, antibiotic concentration and number of organisms were found to affect the development and manifestations of stage two. A slight development of stage two was associated with bacteriostatic effects. Certain marked manifestations of stage two were associated with bactericidal effects.

Fluorescence microscopy of intracellular S. Typhosa showed that the effect of tetracycline was limited to a slight development of stage two. While the living host cell accumulated considerable tetracycline, as evidenced by strong mitochondrial fluorescence, very little appeared to be taken up by the intracellular S. Typhosa. When fluorescence was detectable it appeared predominantly in the wall of the intracellular bacteria.

Melanotic and amelanotic tumor tissue. Melanized and amelanized granules in tumor cells of S91 melanoma were found to stain with tetracycline. Phagocytized melanin, present in compound bodies, on the other hand, did not show fluorescence with tetracycline. This information has been used as one line of evidence for the mitochondrial nature of melanin granules. It was presented at the 5th International Pigment Cell Conference, October 1961.

Tetracycline and liver damage. Attempts to produce fatty degeneration in liver tissue of mice by tetracycline therapy were unsuccessful.

Serial No. NIAID-86A

Significance to Bio-medical Research and the Program of the Institute:

Evidence of early specific effects of virus invasion may indicate an intracellular locus of virus activity and elucidate whether the characteristic cytopathogenic effects are directly or indirectly related to viral production.

Tetracycline provides a visual tag for study of the uptake and localization of an antibiotic and provides further information regarding microbial persistence following antibiotic therapy. In view of the localization of tetracycline in mitochondria of animal tissue cells and the similarity of certain staining patterns of tetracycline to Janus green B in microorganisms, the active material upon which an antibiotic acts in microorganisms may be mitochondrial in nature. Tetracycline has provided further evidence for the mitochondrial nature of melanoma granules.

Proposed Course of the Project:

Work on the cytopathogenic effect on living cells is being continued. A manuscript is in preparation regarding tetracycline and microorganisms. Data on the staining of melanomas has been presented and is to be published.

Serial No. NIAID-86A

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Showacre, J. L., H. E. Hopps, H. G. du Buy and J. E. Smadel. Effect of antibiotics on intracellular salmonella typhosa. I. Demonstration by phase microscopy of prompt inhibition of intracellular multiplication. J. Immunology 87: 153-161, 1961.

Honors and Awards relating to this project:

None

Serial No. NIAID-86B
1. Biology of Viruses
2. Biochemistry & Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Investigations of interference with enzymatic functioning of mitochondria as a mechanism for carcinogenesis

Principal Investigator: Dr. M. L. Hesselbach

Other Investigators: None

Cooperating Units: Laboratory of Pathology, NCI.

Man Years:

Total:	3/12
Professional:	2/12
Other:	1/12

Project Description:

Objectives:

To determine whether interference with the functioning of mitochondrial enzymes leads to neoplasia. Specifically, it is desired to find a single dye which: 1) is adsorbed by mitochondria; 2) effects the functioning of mitochondrial enzymes, and 3) induces neoplasia. Then, perhaps, a connection between these two functions of a single agent can be demonstrated.

Methods Employed:

Conventional Warburg manometry for metabolic study of induced and transplanted tumors, also for analysis of interaction of the dyes under study, with mitochondrial enzymes.

Different methods of preparation of tissue for metabolic studies: slicing, homogenization, and differential centrifugation.

Part B included: No

Chemical analyses for total nitrogen, lactic acid and inorganic phosphorus.

Histological preparation and examination of treated areas, tumors, and organs.

A study of vehicles which would allow repeated injections of Janus green B over a long period has been made. Non-aqueous media were found to be necessary. Prolonged testing has made it possible to choose the best of these.

Major Findings:

Rats, repeatedly injected with Janus green B have developed transplantable tumors at the site of injection.

Significance to Bio-medical Research and the Program of the Institute:

Both viruses (exogenous) and altered subcellular particles (endogenous) have been implicated as the cause of cancer. This study is an effort to determine the relation of mitochondria to these possible oncogenic agents.

Proposed Course of the Project:

Discontinuation of the project because of resignation of the principal investigator. However, the tumors will be further studied, in order to obtain sufficient information for a report to be published.

Serial No. NIAID-86C
1. Biology of Viruses
2. Biochemistry & Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Demonstration of glucose metabolism and peptide bond formation by isolated brain and liver mitochondria

Principal Investigator: Dr. M. L. Hesselbach

Other Investigators: Dr. H. G. du Buy

Cooperating Units: Analytical Chemistry Section, NIAMD

Man Years:

Total:	5/12
Professional:	4/12
Other:	1/12

Project Description:

Objectives:

To demonstrate that isolated mitochondria participate in protein metabolism and that not only isolated brain mitochondria but also liver mitochondria contain the complete enzyme systems to metabolize glucose.

Methods Employed:

Conventional Warburg manometry, chemical lactic acid determinations, centrifugal separation of subcellular elements, biochemical and biophysical approach to choice of materials for a suspension medium which will keep mitochondria structurally and functionally intact, biochemical approach to determining materials to be added to isolated mitochondria to restore *in vivo* enzymatic activity. For demonstration of peptide-bond formation: one-dimensional paper chromatography.

Part B included: No

Major Findings:

A comparison was made between the glucose metabolism of mouse brain and liver mitochondria, and that of Cloudman S_{91} and S_{91} W melanoma granules.

The granule fractions were prepared by the technique of differential centrifugation and by the sucrose gradient technique. It was found that the glucose metabolism of comparable amounts of melanin granules was of the same order of magnitude as that of brain mitochondria. The data were presented at the 5th International Pigment Cell Conference as support for the theory that melanin granules represent modified mitochondria.

Significance to Bio-medical Research and the Program of the Institute:

The relation of viruses to mitochondria (site of virus reproduction, origin of viruses, site of neoplastic change) can only be fully understood when the physical and chemical characteristics of mitochondria become known. It would be strange if liver tissue could not metabolize glucose, since it stores it (as glycogen), etc. It would also be strange if this essential, energy-producing metabolism were not located on the mitochondria in liver cells as it is in brain cells.

If isolated mitochondria can be shown to synthesize the peptide bond, it will add significantly to our knowledge of the role of mitochondria in the living cell.

Proposed Course of the Project:

Discontinuation of the project, with integration of some of the phases with project NIAID-86.

Serial No. NIAID-86D
1. Biology of Viruses
2. Biochemistry & Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Comparison of the properties of crude and crystallized Coxsackie A-10 virus, of Cloudman S_{91}, mouse melanoma and of mouse muscle nucleoprotein

Principal Investigator: Dr. H. G. du Buy

Other Investigators: H. Sasame

Cooperating Units: None

Man Years:

Total:	6/12
Professional:	2/12
Other:	4/12

Project Description:

Objectives:

To compare preparations of purified virus with those of mouse muscle and melanoma nucleoprotein, chemically and immunologically.

Methods Employed:

For virus purification: Coxsackie A-10 virus of suckling mouse origin is purified and concentrated by chemical, physical and ultracentrifugal means. Purified virus is analyzed for protein and nucleic acid content.

For muscle and S_{91} nucleoprotein preparation available methods were not applicable to the materials under study. Some steps, used for the preparation of so-called ribosomes, followed by modifications of existing purification procedures, have given promising results.

Part B included: No

Serial No. NIAID-86D

Major Findings:

The intracellular distribution of ribose nucleic acid over subcellular fraction of five tissues was compared. The fractions were obtained by selective centrifugation. The tissues investigated were: mouse liver, mouse brain, S_{91} mouse melanoma, and the $S_{91}W$ amelanotic mouse melanoma (derived from the S_{91} melanoma).

The amount of RNA in the melanoma granules was of the same order as that found in the granules of the amelanotic melanoma and that in the mitochondrial fraction of the other tissues. This was used as one of the five lines of evidence, that melanin granules are modified mitochondria. The data were presented at the 5th International Pigment Cell Conference, October 1961.

The introduction of viruses into normally resistant cells by cellular uptake of glass-adsorbed virus has been further studied. Further studies are necessary to determine whether this uptake was due to phagocytosis or to mechanical introduction by centrifugal force.

The introduction of the self-duplicating portion of melanoma cells into susceptible hosts has led to negative results, thus far. No satisfactory method for the preparation of active nucleic acid is yet available.

Significance to Bio-medical Research and the Program of the Institute:

The introduction of virus into cells which are normally not susceptible to this virus, except in its nucleic acid stage, might throw further light on the conditions which govern virus multiplication in host cells.

The introduction of a self-duplicating portion of mitochondria from cancer cells into susceptible hosts might lead to formation of this specific neoplasm, and thus bridge the virus and the mitochondrial theories of carcinogenesis.

Proposed Course of the Project:

Discontinuation of the project. Parts to be incorporated into project NIAID-86.

NIAID 87
Laboratory of Biology of Viruses
Section on Cell Biology
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: The Mechanism of Production of Poliovirus in Cultured Mammalian Cells

Principal Investigator: Dr. Leon Levintow

Other Investigators: Dr. Jacob V. Maizel, Jr., Dr. Matthew D. Scharff, Mrs. Marilyn M. Thoren, Mr. J. Leonard Hooper and Mr. Aaron E. Freeman

Cooperating Units: None

Man Years (calendar year 1961)
Total: 8-6/12
Professional: 2-6/12
Other: 6

Project Description:

Objectives: To define the events during the adsorption and maturation of poliovirus in biochemical terms, and to investigate the mode of replication of viral protein and RNA.

Methods Employed: Cultured cells are infected with poliovirus in a chemically defined medium, and the course of the infection is followed by quantitative plaque assays of infectious virus and infectious RNA. Virus isolated from infected cultures is purified, degraded, and characterized chemically by a variety of procedures, including chemical and enzymatic hydrolysis, paper and column chromatography, electrophoresis, and isotope tracer techniques.

Major Findings: The fact that poliovirus is assembled from the soluble amino acids and nucleotides of the cell has permitted study of the formation of the viral protein and nucleic acid with the aid of radioactive isotopes. These results, confirmed by independent techniques, indicate that the synthesis of the two macromolecules is closely coordinated in time, and suggest the possibility that their replication may be interdependent. Further insights into this problem have been gained by utilizing metabolic antagonists. Although viral RNA can be formed in the presence of a concentration of

Part B included

p-fluorophenylalanine which prevents formation of infectious virus, the results do not necessarily imply dissociation of virus protein and nucleic acid under these conditions. Moreover, the results with puromycin, a specific inhibitor of protein synthesis, which prevents virus nucleic acid synthesis as well, support the view that replication of the latter depends on prior or concomitant specific protein synthesis.

Poliovirus appears to consist of a single molecule of ribonucleic acid, enclosed in a protein coat of 60 apparently identical subunits. Chemical and physical determination of the size and character of the subunit has been undertaken, data which have not been available for any animal virus. The purification procedure has been modified to permit the weekly production of 6-10 mg of pure virus for study. As is the case with several plant viruses, no free N-terminal amino acid residues has been found. Preliminary estimations of the size of the basic chemical subunit indicate that it is considerably larger than the predicted unit molecular weight of about 20,000.

Significance to the Program of the Institute: Study of virus replication not only affords insights into the pathogenesis of viral disease, both at the cellular and whole animal level, but provides a useful model of a rapidly reproducing biological unit at the cellular and sub-cellular level. The system *in vitro* is well adapted for the investigation of general problems concerning the mode of replication of protein and nucleic acid.

Proposed Course of the Project:

1. The synthesis of specific protein in poliovirus infected cells and its relationship to nucleic acid synthesis, will be investigated by sensitive and specific immunological techniques.

2. Studies on the chemical and physical characterization of the poliovirus subunit will be continued.

3. Further studies will be carried out on the chemical, physical and biological properties of the infectious RNA extracted from purified virus.

4. Attempts will be made to develop a practical system for the study of aspects of viral replication at the sub-cellular level.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Levintow, L., Thoren, M. M., Darnell, J. E., Jr., and Hooper, J. L. Effect of p-Fluorophenylalanine on the Replication of Poliovirus. Virology. In press.

Levintow, L. and Eagle, H. The Biochemistry of Cultured Mammalian Cells. Ann.Rev.Biochem. 30: 605-640, 1961.

Darnell, J.R., Jr., Levintow, L., Thoren, M. M. and Hooper, J. L. The Time Course of Synthesis of Poliovirus RNA. Virology 13: 271-279, 1961.

Joklik, W. K. and Darnell, J. E., Jr. The Adsorption and Early Fate of Purified Poliovirus in HeLa Cells. Virology 13: 439-448, 1961.

Darnell, J. E., Jr. The Origin and Time of Synthesis of Constituents of Poliovirus. Perspectives in Virology, Vol. 2.

Levintow, L. and Eagle, H. The Technique of Mammalian Cell Culture. In Colowick and Kaplan's Methods in Enzymology, Vol. 5. In press.

Darnell, J. E., Jr. and Eagle, H. The Biosynthesis of Poliovirus in Cell Cultures. Adv. in Virus Research, Vol. 7, 1-26, 1960.

Honors and Awards relating to this project:

Invited lecture: "The Biochemistry of Poliovirus," at Tufts University, School of Medicine, Department of Biology, May 12, 1961.

NIAID 88
Laboratory of Biology of Viruses
Section on Cell Biology
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Mechanism of Drug Resistance in Cultured Mammalian Cells

Principal Investigator: Dr. Stanley Barban

Other Investigators: Mr. Henry O. Schulze

Cooperating Units: None

Man Years (calendar year 1961)

Total:	3
Professional:	1
Other:	2

Project Description:

Objectives: To study the biochemical basis of the development of resistance to a glucose analogue in a strain of human cells in culture; to determine the effect of various hormones on drug resistance; and to investigate the glucose transport system in this drug resistant strain.

Methods Employed: The techniques employed in this project are: cell fractionation and isolation of enzymes of parent sensitive and drug resistant cells; use of various chemical and enzymatic procedures in assaying for carbohydrates and their metabolic products; and use of isotopically labeled precursors. These procedures have been described in detail in previous publications relating to this project.

Major Findings:

1. Biochemical Basis of Resistance. Previous studies have demonstrated that resistant cells have a reduced capacity to phosphorylate and transport the antimetabolite, 2-deoxy-d-glucose, into cells. This altered transport mechanism for the glucose analogue has been found to be related to the presence of an inhibitor of hexose phosphorylation in the resistant cells. Another significant finding is an 8-10 fold higher level of alkaline phosphatase activity in resistant cells as compared to the parent sensitive cells. The question of whether the inhibitor of hexose phosphorylation is identical with alkaline phosphatase is under current study.

Part B included

2. Effects of Hormones on Drug Resistance. Recent findings of Cox and MacLeod have shown that prednisolone, a hydrocortisone analogue, induces alkaline phosphatase activity in some human epithelial cells. We have attempted to increase alkaline phosphatase activity in sensitive cells as a mechanism of decreasing sensitivity to the toxic effects of 2-deoxyglucose. It has been found that when sensitive cells are grown in the presence of prednisolone, they are indeed capable of circumventing the toxic effects of 2-deoxyglucose. This finding suggests that one mechanism by which resistance to the antimetabolite can be effected is a consequence of higher levels of the enzyme alkaline phosphatase.

Significance to the Program of the Institute: The development of resistance to metabolite analogues is a serious problem in the therapy of neoplastic diseases as well as infectious diseases. An understanding of the nature and mechanisms of the biochemical changes leading to resistance to metabolite analogues and other drugs poses fundamental questions in areas of chemotherapy, enzymology and biochemical genetics.

Proposed Course of Project:

1. The nature of the inhibitor of hexose phosphorylation in resistant cells is being studied in connection with its relationship to alkaline phosphatase. In addition, the effect of hormones on the induction of the enzyme alkaline phosphatase and its relationship to drug resistance will be further explored.

2. Studies are under way to transform drug sensitive cells to drug resistance, using the DNA from deoxyglucose-resistant cells. This system will also be used to study the genetics of drug resistance.

3. Since the resistant cells have an altered hexose transport system, this strain of cells will be used to study the problem of carbohydrate transport in mammalian cells. The relationship of various hormones, i.e. insulin to carbohydrate transport will be investigated.

4. The relationship of glucose metabolism to viral synthesis employing the resistant strain, is under study.

5. The resistant cell line has been found to require pyruvate for sustained growth in the regular cell culture medium. In view of the key role of pyruvate in cell metabolism, the importance of this metabolite for the nutritional requirements of drug resistance will be studied.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Barban, S. Mechanism of Resistance to 2-Deoxyglucose in HeLa Cells. Biochim. et Biophys. Acta 47: 604-605, 1961.

Barban, S. and Schulze, H.O. The Effects of 2-Deoxyglucose on the Growth and Metabolism of Cultured Human Cells. J.Biol.Chem. 236: 1887-1890, 1961.

Barban, S. Studies on the Mechanism of Resistance to 2-Deoxyglucose in Mammalian Cell Cultures. In press.

Honors and Awards relating to this project: None.

Serial No. NIAID-89
1. Biology of Viruses
2. Biochemistry & Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Kinetics and Sites of Coxsackie Virus Multiplication

Principal Investigator: Dr. C. F. T. Mattern

Other Investigators: Lotta Chi
Wendell Daniel

Cooperating Units: None

Man Years:
Total: 2 4/12
Professional: 1
Other: 1 4/12

Project Description:

Objectives:

To establish the intracellular site or sites and mechanism of viral RNA and protein synthesis.

Methods Employed:

One phase of this study has been essentially completed. Coxsackie A-9 virus was cultivated in monkey kidney cell and samples of cells harvested at various time periods after inoculation. The cells were separated into "cytoplasmic" and "nuclear" fractions by citric acid homogenization and centrifugation. These two fractions were studied for their content of mature virus. Additionally, they were extracted with cold phenol, which does not split mature Coxsackie A-9 virus, to determine their infectious RNA content.

A second phase of this investigation involves following these same events with fluorescent antibody studies. High titering antibody to purified Coxsackie A-9 virus was prepared and is used with the indirect fluorescent antibody technique to attempt to localize sites of viral antigen synthesis.

Part B included: No

Major Findings:

1. The distribution of infectious RNA between the "nuclear" and "non-nuclear" fractions was found to vary during the cycle of infection. During the early stages, between one and three hours, the total extractable infectious RNA, as measured by cold phenol treatment of whole cells and the citric acid homogenate, appeared to be associated with the "nuclear" fraction. Beyond three hours, the infectivity was associated with the "non-nuclear" fraction. The total increase in infectivity of RNA in the "non-nuclear" fraction was about 8,000-fold, whereas that of the "nuclear" fraction was about 90-fold.

On the other hand, the distribution of mature virus between the same two fractions showed that whole virus was higher in the "non-nuclear" fraction at all stages of the cycle.

These data have been interpreted in the following way. Invading virus releases its RNA within the host cell, after which this RNA becomes associated with the cell nucleus. Whether this association is specifically related to viral multiplication or an incidental association due to acidophilic properties of the nucleus cannot be answered. Subsequently, and as early as two hours after infection, there is an increase in infectious RNA in the "non-nuclear" fraction, presumably representing new nucleic acid synthesis in some cytoplasmic element. This multiplication proceeds at about the same rate and for approximately the same length of time, as does the accumulation of newly synthesized mature virus. Thus, these data are felt compatible with the hypothesis that invading virus RNA may initially induce some nuclear alteration as a result of which the cytoplasm initiates viral RNA replication and possibly protein synthesis as well.

2. Fluorescent antibody studies have shown an initial faint cytoplasmic staining at about 1 hour, decreasing to almost no staining at 3 hours. Thereafter, there is a rapid increase of cytoplasmic staining. This fluorescent staining distribution closely parallels the mature virus concentration found in the cytoplasmic fraction. To date, no evidence of a second "nuclear" antigen has been found.

Significance to Bio-medical Research and the Program of the Institute:

This study is intended to contribute to our knowledge of the nature of the processes by which viruses multiply, the ultimate goal of which is to find rational means of controlling virus infections.

Proposed Course of the Project:

It is hoped that the events described can be followed by high resolution electron microscopy of virus-infected cells. By this means, it may be possible to confirm the proposed hypothesis.

Additional studies are planned to elucidate the physical and chemical nature of the virus precursor present during the early stages of virus infection. Of particular interest is the question as to whether this precursor is a ribonucleoprotein or RNA alone in a configuration which renders it stable to ribonuclease.

Serial No. NIAID-89A
1. Biology of Viruses
2. Biochemistry & Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Virus Structure

Principal Investigator: Dr. Carl F. T. Mattern

Other Investigators: Dr. Arthur G. Malmon
Lotta Chi

Cooperating Units: None

Man Years:

Total:	1	10/12
Professional:	1	
Other:		10/12

Project Description:

Objectives:

To elucidate the structure of animal viruses.

Methods Employed:

1. High resolution electron microscopy employing a variety of techniques including conventional metal shadowing, negative staining, positive staining and new methods of controlled metal shadowing.

2. X-ray diffraction. Hypothetical models of virus are constructed and theoretical X-ray diffraction patterns calculated and compared with X-ray patterns obtained by other investigators. Arrangements have been made to obtain X-ray pictures of virus crystals where such are not available.

3. Analytical ultracentrifugation is used to determine the size of various virus components.

4. Chemical analyses are performed including nucleic acid base composition. Amino acid composition will be determined.

Part B included: No

Serial No. NIAID-89A

Major Findings:

1. Coxsackie virus has been shown to have an outer envelope, presumably protein, and a dense inner core of RNA or ribonucleoprotein.

2. A "top" component in the cesium chloride gradient has been found to consist largely of hollow "spheres," presumably empty protein shells, containing relatively little infectivity. This component is analogous to the top components found with poliovirus and turnip yellow virus.

3. Coxsackie virus has been found to contain about 25% RNA by weight and chemical analysis of the RNA of Coxsackie virus groups A-9 and A-10 show them to be essentially identical, but both different from the base composition of poliovirus.

4. A model has been constructed which may explain the discrepancies between X-ray diffraction deduced structure of tobacco mosaic and the observations of electron microscopy.

Significance to Bio-medical Research and the Program of the Institute:

This project is an effort to elucidate the 3-dimensional structure of animal viruses; their subunit orientation, subunit substructure, and the configuration of the RNA within the virus.

Proposed Course of the Project:

To continue to investigate by the means described four known structures associated with Coxsackie virus: the mature virus, top component, virus precursor, and ribonucleic acid as extracted by the hot phenol procedure.

Serial No. NIAID-89B
1. Biology of Viruses
2. Biochemistry & Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Methods for Study of Nucleic Acid Structure

Principal Investigator: Dr. Arthur G. Malmon

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 5/12
Professional: 5/12
Other:

Project Description:

Objectives:

To develop new techniques for the study of nucleic acid structure.

Methods Employed:

The particular methods to be used are in the planning stage. The general procedure will be to label specific regions of a ribonucleic acid molecule with a fissionable material, deposit the molecule in extended form on a material suitable for electron microscope observation, induce fission in the fissionable atoms by exposure to an appropriate neutron source, and, by observing the tracks of the fission fragments by electron microscopy, determine the relative location of the labeled site.

Major Findings:

No experimental work has yet been performed. However, the feasibility of observing fission tracks in a suitable substrate, and of inducing sufficient fission events in a reasonable time with available neutron sources has been established.

Part B included: No

Serial No. NIAID-89B

Significance to Bio-medical Research and the Program of the Institute:

If successful, this method will offer a means of relating chemical properties, and possibly genetic characteristics, to position on a nucleic acid molecule. Such information will contribute to the understanding of the biological function of nucleic acids.

Proposed Course of the Project:

A prime requirement for application of this method is the ability to attach the fissionable atoms to specific location on the nucleic acid molecule. Theoretical and experimental investigation of this problem will be undertaken; the course will be to analyze the available data on binding of metal ions to nucleic acid, and to search for conditions which promote meaningful binding of a fissionable material, particularly uranium.

LABORATORY OF TROPICAL VIROLOGY
Middle America Research Unit
Arthropod-Borne Virus Section

Summary 1

93 - Studies on the Epidemiology of Arthropod-Borne Virus Infections 5
94 - Study of Arthropod-Borne Viruses in Tissue Culture 8
95 - Development of Experimental Procedures to Improve Serological Detection of Arthropod-Borne Virus Infections 10
96 - Grouping of Arthropod-Borne Viruses with Polyvalent Antiserum Pools 12
97 - Studies on Newly Recognized Groups of Arthropod-Borne Viruses. (1) The Bunyamwera Group 14
99 - Studies on Attraction and Feeding Behavior of Biting and Bloodsucking Arthropods in Relation to Preventive Measures 17
100-A- Epidemiological Investigations and Surveillance of Infectious Disease Situations in Panama and Central America 20
100-B- A Clinical and Virological Study of Oropharyngeal Lesions in Panamanian Children 26
100-C- Venezuelan Equine Encephalomyelitis in Panama 28
100-D- Serological Investigation of Measles Virus in Isolated Populations (in Panama) 32
100-E- Vesicular Stomatitis Virus (VSV) Infections in Panama 34
101-A- Virological Aspects of a Cooperative Investigation on the Ecology of Arthropod-Borne Viruses 37
101-B- An Appraisal of Chiggers and Other Acarina of the American Tropics for Pathogenic Agents 42
101-C- Viral "Jungle Fever" in U.S. Military Personnel 44
102-A- Intestinal Viral Flora in Rural Guatemalan Children A Survey of Three Villages 46
103-A- Use of Filter Paper Discs for Virus Isolation and Serological Testing 49
105-A- Studies of Microbial Diseases of Military Importance in Latin-America 53
108 - Studies of Histoplasmosis on the Isthmus of Panama . 57
109 - Studies of Superficial and Deep Mycoses in Panama and Central America 63

PHS-NIH
Summary Statement by Chief
Laboratory of Tropical Virology
Calendar Year 1961

ADMINISTRATIVE ASPECTS

The third year of the existence of the Laboratory was as eventful and stormy as the last, with administrative upheavals, personnel shifts and redirection of research programs.

In mid-year Dr. Shelokov relinquished directorship of Middle America Research Unit (MARU) to Dr. Beye and Dr. Peralta replaced him as Acting Head of the Virus Diseases Section. Major Diercks replaced Major Taylor as Head of the redesignated Mycotic and Bacterial Diseases Section. Dr. Beye organized the Epidemiology Section, which besides Drs. Brennan and Yunker (the former Acarology group) includes Dr. Mackenzie, who recently reported on duty. Dr. Ordonez completed his one-year fellowship in virology and departed for Pittsburgh. Dr. Brody was transferred to Bethesda, pending a 6 months' assignment to Moscow in 1962. Dr. Bruckner, Dr. Peralta and Mr. Vogel contracted Venezuelan encephalitis in the course of studies on what eventually turned out to be a major urban epidemic of this disease; all the MARU personnel received live VEE virus vaccine; for many months field and laboratory VEE studies replaced most of the scheduled virological activities.

A natural shift in interests has occurred with Dr. Shelokov's return to Bethesda and the addition of Drs. Wiebenga and McCloskey plus several new technical personnel to the Arthropod-borne Virus Section staff. Mr. Gibbs, Dr. Woke and Mr. Pennington have remained with the Section.

The physical facilities of both laboratories changed during the year: MARU expanded to the second and third floors of the

former Canal Zone nurses home, while the dismal basement of Building 5 at NIH was rebuilt into an attractive modern laboratory (in addition, two office and three laboratory rooms on the second floor and an attic space for uninfected animals were assigned to LTV). Reconstruction and expansion interferred with work in both locations, but the Bethesda laboratory had to be shut down for about two months of the year!

The administrative and personnel changes noted for MARU have not radically altered the virological research program (except for epidemic emergency needs), but new interests in specific parasitic and bacterial diseases are bound to emerge. In Bethesda, the mission and the objectives of the Section have been redefined, research projects redesigned and new ones initiated. The planned program will relate the work of the two virus sections, coordinating the field and laboratory investigations on arthropod-borne viruses of public health importance.

We hope that the next year will be less stormy, although it is bound to be eventful; for one thing, we still have to fill the two critically important vacancies of the virus section Heads in Panama and in Bethesda.

RESEARCH HIGHLIGHTS

During the period of about four months, devoted to program reorganization, recruitement and training of technical personnel, the Bethesda group has not had the time to develop enough new material to be featured here. On the other hand, the Canal Zone component has continued its productive activities along the established lines, but with many new findings.

Venezuelan Equine Encephalitis (VEE). The first isolation of this virus in Panama was accomplished in April from specimens obtained from a dying Panamanian boy. Three senior laboratory staff members became infected as a result of the exposure (with virus isolation and serological confirmation in each case). Entire MARU contingent was then immunized with the live attenuated VEE virus vaccine. Mild reactions were often encountered, and the attenuated virus was isolated from some of the vaccinees. Several aspects of practical application of this still experimental preparation were thus explored.

Epidemiologic and ecologic studies of the rural community where the case had occurred (in collaboration with Gorgas Memorial

Laboratory), failed to demonstrate the VEE virus in wild, domestic or sentinel animals, or the mosquitoes collected. Serological evidence of VEE infection (CF, HI, NT) was established for a significant percentage of residents of the area.

A remarkable urban epidemic of VEE occurred in another part of Panama near the site of the Gorgas Memorial Laboratory field station. Approximately 350 cases were registered during a two months' period. Important new information on the epidemiology and the clinical spectrum of epidemic infection with this virus is being developed by GML and MARU.

Vesicular Stomatitis Virus (VSV). Last year we reported repeated isolation of Indiana type VSV from phlebotomus sandflies collected in the course of a collaborative project with Gorgas Memorial Laboratory's on the ecology of arthropod-borne viruses. Approximately 700 human sera were collected from the population of a town near the field station. One-fourth of the 490 sera tested had neutralizing antibodies to Indiana type VSV. One-fourth of the neutralizing sera also possessed CF antibodies.

Our interest in this virus prompted us to investigate an outbreak of vesicular stomatitis in cattle. The causative agent was found to be the New Jersey, rather than Indiana, type of VS virus. Antibody patterns of humans in close or remote association with the infected cattle suggested that direct contact, rather than arthropod vectors, may be involved in the transmission of this infection to man. It would seem that the ecology and epidemiology of the two types of VSV are quite dissimilar.

Virus Isolations from Rain Forest Arthropods. During the second year of the study on the ecology of arthropod-borne viruses in the tropical rain forest of Panama, conducted by GML (NIH grant E-2984) in collaboration with MARU, the number of virus isolates was doubled: present total 38 - 14 from mosquitoes and 10 from phlebotomus sandflies. The isolation rate from sandflies continues to exceed by far the overall isolation rate from mosquitoes.

The first isolate of Indiana VSV from a *non*-sandfly source was recorded (mosquito *Culex nigripalpus*). Many of our mosquito isolates have been identified with collaboration of the Trinidad and Belem laboratories of the Rockefeller Foundation: six strains closely related to *Una* virus of Group A (work with four other similar agents has not been completed); one strain of Ilheus virus of Group B; several members of the *Bunyamwera* group-one *Guaroa*

and four Wyeomyia viruses; Guama group, represented by six isolates, all from the same mosquito (Culex vomerifer). A few other agents remain unidentified.

Ascitic Fluid as a Serologic Reagent. Mice were immunized with virus plus an ascites-stimulating adjuvant mixture. The resulting antibody-laden ascitic fluid has been useful in CF tests, particularly for relating isolates to one another and to prototype viruses, and has to a great extent replaced the mouse immune serum.

Failure to Recover Viruses from Parasitic Mites. Nearly 500 pools of acarines (including 25 species of chiggers, 35 of parasitoid mites and a few ticks from over 350 wild vertebrates) were inoculated into suckling mice and hamster kidney cell cultures. Not a single viral agent was isolated, attesting to the uncommon presence of at least the recognizable viruses among the common tropical mites. Many ectoparasite pools were shipped to the Rocky Mountain Laboratory for attempted recovery of rickettsia. One positive isolation of the C. burnetii-like agent was made from a species of intradermal chigger, parasitic on the tropical spiny rat.

Filter Paper Discs as Epidemiological Field Tool. The use of blood-soaked discs for the isolation of arthropod-borne viruses from birds and small mammals has been disappointing in our hands. However, they appear useful in screening (NT & HI) the hard to bleed infants and small animals for antibodies against arthropod-borne and enteric viruses.

Histoplasma capsulatum from Bat Organs. Repeated isolation of this fungus from bat liver and spleen suspensions was a most important finding, providing further evidence for the suspected role of bats in the ecology of histoplasmosis.

Actinomycosis in Panama. The first human case of actinomycosis (cervical-facial type) caused by A. bovis was recorded, demonstrating the existence of this disease on the Isthmus of Panama.

Serial No. NIAID - 93
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Studies on the epidemiology of arthropod-borne virus infections

Principal Investigator: Dr. A. Shelokov

Other Investigators: C. J. Gibbs, Jr., Dr. N. Wiebenga, Dr. R. McCloskey and R. M. Pennington

Cooperating Units: Dr. D. Mendez-Cashion, Dept. of Pediatrics, U. of Puerto Rico

Dr. J. Ehrenkranz, Dept. of Med., U. of Miami

Dr. D. C. Gajdusek, NINDB, NIH

Dr. A. Matson, The Minneapolis War Memorial Blood Bank, 2304 Park Avenue, Minneapolis 4, Minnesota

Dr. C. J. Gibbs and Dr. R. J. Byrne, Livestock Sanitary Service, U. of Md.

Southwest Blood Banks, Inc.

Middle America Research Unit, Panama Canal Zone

Man Years (Calendar year 1961)

Total:	2 1/4
Professional:	3/4
Other:	1 1/2

Project Description:

Objectives:

To elucidate the role of selected arthropod-borne viruses as causes of infection of human and animal populations in the Western hemisphere.

Part B included - No

Methods Employed:

Whenever clinical materials from sporadic cases or from epidemic outbreaks are available, virus isolation attempts are made; if a viral agent is isolated, it is related to the illness by antibody studies on the serial blood specimens; it is also characterized and identified. The prevalence of specific antibodies in the population to unusual or apparently important viruses is established. Selected viruses from our Canal Zone laboratory and outside organizations are used in neutralization, HI and CF tests against the representative sera from the geographic areas of concern, to determine the prevalence or the relation of the infection to clinical disease.

Major Findings:

This long-term project has just begun. The virus strains have been collected from many investigators for preparation of antigens and antisera and serum collection has been set up for selective testing. The initial scope of these activities and few of the available results are outlined.

I. United States. During the year virus isolation attempts from U. S. specimens were limited to blood and spinal fluid from 2 horses dying from encephalitis in near-by Maryland in October 1961 (cooperation of Livestock Sanitary Service, U. of Md.). No viruses were isolated although confirmed cases of Eastern equine encephalitis were occurring in Maryland and New Jersey at this time.

Preliminary baseline studies on the experience of human populations of southern United States (Texas and Florida) with group A and group B viruses were carried out. Hemagglutination-inhibition and neutralization test results indicate activity of Eastern and Western equine encephalitis (group A), St. Louis encephalitis and possibly Ilheus viruses (group B). These North American sera, to be supplemented by other U. S. collections, will be tested for several apparently important viruses recently isolated by our Canal Zone laboratory.

II. Middle and South America. With the collaboration of Dr. Mendez-Cashion, 192 blood serum specimens were obtained from 121 pre-school children hospitalized in Puerto Rico with acute neurological illnesses of obscure etiology. Approximately half of these were admission specimens suitable for virus isolation attempts. Some specimens of throat swabs, urine and autopsy materials are also available. Should viral agents be isolated, they will be related to the illness of individual patients by testing of paired serum specimens. Population experience will be studied with

the growing collection of blood specimens from life-long residents of the Island representing age groups from 15 to 65 years (150 sera so far collected).

Because we adhere to a policy of not importing "foreign" viruses to the Isthmus of Panama, the Canal Zone laboratory cannot test for serological evidence of infection with certain agents which may be active in this part of Central America. We are preparing to provide these answers by testing the Panamanian sera in Bethesda.

A total of 273 Guatemala sera had been screened for antibody patterns with group A and B antigens. Evidence of previous infection with Eastern and Western equine encephalitis (group A) and yellow fever, St. Louis encephalitis and Ilheus viruses (group B) was demonstrated. Notably, the antibody pattern was similar to that established for the southernmost United States with the exception, as would be expected, of the yellow fever antibodies in Guatemala.

With the collaboration of several field operating organizations (particularly through Dr. Matson and Dr. Gajdusek) a large serum collection from indigenous (especially Indian) populations of Mexico, Honduras, Nicaragua, Costa Rica and Peru is now on hand.

Gradually arthropod-borne viruses from other parts of the world and sera from known areas of activity are being accumulated. Among them are the still not completely identified recent Australian virus isolates and serum specimens from the Australian and New Guinea aborigines.

Significance to bio-medical research and the program of the Institute:

A study of the epidemiology of the arthropod-borne viruses is necessary to understand their relative importance and constitute a prerequisite to the attempted control of the infection and disease by prophylaxis and other means.

Proposed Course of the Project:

The studies will be broadened to include geographically important areas of known and suspected arthropod-borne virus activity. Still closer coordination with our Middle America Research Unit, the Rocky Mountain Laboratory of NIAID and certain outside agencies engaged in similar activities is envisaged to provide a coordinated attack on the complex problem of epidemiology and ecology of these agents.

Serial No. NIAID - 94
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Study of arthropod-borne viruses in tissue culture

Principal Investigator: Dr. Ned H. Wiebenga

Other Investigators: Dr. R. V. McCloskey

Cooperating Units: None

Man Years (Calendar year 1961)
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

(1) Establishment of a collection of viruses harvested from susceptible tissue culture systems, (2) development of wild and TC adapted antigens in suitable cultures least contaminated with other antigens, (3) characterization of viruses in susceptible TC systems, (4) development of TC protocols to replace animal assay systems whenever sensitivity, economy, or efficiency can be improved, and (5) development of attenuated virus strains useful for production of competent and safe vaccines.

Methods Employed:

Biological responses of promising TC systems are assayed for comparison with standard assays established by recognized investigators. Assay evaluation is based on infectivity, pathogenicity, antigenicity, population analysis and variation of genetic markers.

Part B included - No

Major Findings:

Work during this reporting period primarily concentrated on the production of Bunyamwera group virus yields in monkey stable (MS) cell culture line. All 8 members of the group have provided concentrated virus yields with complete cytologic destruction (CPE) within 72 hours or less. Assay of terminal dilutions by CPE seems to be complete within 5 days after inoculation. Results are incomplete but promising of comparable sensitivity and yields with those obtained in suckling mice. Furthermore, the potential of antigenic superiority is suggested by the concentration of virus in a simpler menstruum than mouse brain suspension.

Significance to bio-medical research and the program of the Institute

The importance of the stated objectives of this project in the development of a high caliber virus laboratory devoted to tropical viruses, particularly arthropod-borne, is self evident. Selection of Bunyamwera as the initial virus group to be subjected to this type of investigation is apparent from reference to the accompanying individual project report (NIAID - No. 97) on the comprehensive studies on the several members of this virus group initiated by the Section during the calendar year.

Proposed Course of the Project:

Upon completion of characterization studies in MS cell system, purification of viruses by plaque technique will be possible. The experimental design will be used as a model for similar studies with other arthropod-borne virus groups selected because of their potential public health importance.

Serial No. NIAID - 95
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Development of experimental procedures to improve serological detection of arthropod-borne virus infections

Principal Investigator: Dr. A. Shelokov

Other Investigators: Dr. R. McCloskey and R. M. Pennington

Cooperating Units: Dr. J. L. Sever, NINDB

Man Years (Calendar year 1961)

Total:	2 1/4
Professional:	3/4
Other:	1 1/2

Project Description:

Objectives:

To develop (by designing or adapting) additional experimental procedures needed to improve detection of serum antibody responses in the study of pathogenesis, immunology, epidemiology and ecology of known and newly recognized arthropod-borne virus infections.

Methods Employed:

During the year 3 experimental procedures were employed: (1) Takatsy's spiral loop microtitrator technique of HI and CF tests for arthropod-borne viruses, (2) kinetic neutralization technique and (3) bentonite flocculation test (see 1960 Individual Project Reports Nos. 95a and 95b respectively). In addition a simplified procedure for the performance of CF tests has been worked out.

Part B included - No

Major Findings:

(1) Comparison of the Takatsy spiral loop technique with the standard plate method of performing HI tests with several arthropod-borne viruses showed it to be internally consistent, yielding reproducible and comparable results. (2) Kinetic neutralization test continues to show promise in the differentiation of homologous and heterologous antigen-antibody responses, although further modifications in the test procedure will be required. (3) Bentonite flocculation test gave apparently specific results when a protype strain of Eastern equine encephalitis virus, grown in hamster kidney cell culture, was used. However, continuing variability in the virus titers has been so marked with different lots of HKTC fluid that studies with this test system will be discontinued as impractical.

A procedure has been worked out for simplified preparation of potent and specific CF antigens for group A viruses by rendering simple borate stock buffer suspensions non-infectious with ethylene oxide gas.

Significance to bio-medical research and the program of the Institute:

The so-called "standard" techniques of CF and HI tests for serum antibody detection often are not available or not readily applicable for certain arthropod-borne viruses, especially recent isolates. Development of adequate serological tools is a prerequisite to a thorough study of the virus and its role in the causation of disease.

Proposed Course of the Project:

The Takatsy microtitrator technique now will be used routinely in this laboratory together with the standard HI and CF techniques. The kinetic neutralization test will be employed in the special studies on the importance of the "fresh serum factor" (in collaboration with the U. of Md.). The bentonite flocculation test will be abandoned because of lack of reproducibility of results under present conditions. Other techniques will be either developed or applied to arthropod-borne virus work as the need arises.

Serial No. NIAID - 96
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Grouping of arthropod-borne viruses with polyvalent antiserum pools

Principal Investigator: Dr. A. Shelokov

Other Investigators: C. J. Gibbs, Jr., Dr. R. V. McCloskey, Dr. N. H. Wiebenga and R. M. Pennington

Cooperating Units: Dr. W. F. Scherer, Univ. of Minnesota

Dr. R. Shope, Belem Virus Laboratory, Brazil

Dr. J. Casals, Rockefeller Foundation Virus Laboratory, New York, N. Y.

Man Years (Calendar year 1961)

Total:	1 3/4
Professional:	3/4
Other:	1

Project Description:

Objectives:

To design experimental procedures for preparation of polyvalent antisera and to evaluate their usefulness as standard reagents in grouping known and newly isolated arthropod-borne viruses.

Methods Employed:

In the first phase of this project monotypic antisera are prepared by multiple injections of animals with virus infected mouse brain or cell culture suspensions. The sera are tested for CF, HI and neutralizing antibodies

Part B included - No

to the homologous and to heterologous but antigenically related viruses. Combination antiserum pools, prepared by mixing 2 or more monotypic antisera of the same serological group, are tested for antibodies reacting with all available antigens of each virus group.

Major Findings:

Group A arthropod-borne viruses elicited high levels of relatively specific neutralizing antibodies in rabbits and horses. Low order heterologous neutralization by monotypic sera occurred only when the test virus was a member of the same antigenic sub-group as the virus used to immunize the animal. A broadly reactive antiserum pool, found suitable for grouping all group A viruses thus far tested, was prepared by including at least one monotypic antiserum representative of each of the antigenic subgroups.

Significance to bio-medical research and the program of the Institute:

This is a cooperative study among the members of the Sub-Committee on Serological Reagents of the American Committee on Arthropod-borne Viruses. The Sub-Committee will select the most suitable and practical experimental protocols for preparation of grouping and typing antisera, as part of the standard reference and diagnostic reagent program. The Institutes have recognized standard reagents as a major need of virus research laboratories in the United States.

Proposed Course of the Project:

The use of combined antiserum pools for grouping of viruses by complement fixation and hemagglutination-inhibition tests is being explored. As part of the second phase, methods for the production of polyvalent immune ascitic fluids in large and small laboratory animals (in place of antisera) have been developed. Work has begun to determine (1) optimal methods for ascitic fluid production in suitable hosts and (2) the nature of responses to monovalent and/or polyvalent antigens administered serially or in combination mixtures. Initial emphasis is on the production of ascitic fluids in guinea pigs for the Bunyamwera group and selected group B viruses. Whenever possible viruses grown in cell culture will be employed to reduce the possibility of (a) iso-allergic reactions to brain material and (b) formation of non-specific mouse brain antibodies in guinea pigs.

Serial No. NIAID - 97
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Studies on newly recognized groups of arthropod-borne viruses. (1) The Bunyamwera group

Principal Investigator: Dr. A. Shelokov

Other Investigators: Dr. N. H. Wiebenga, C. J. Gibbs, Jr., C. R. Rosenberger, Dr. R. V. McCloskey and R. M. Pennington

Cooperating Units: None

Man Years (Calendar year 1961)
Total: 1 1/2
Professional: 1
Other: 1/2

Project Description:

Objectives:

To define: (a) the pathogenesis of newly recognized groups of arthropod-borne animal viruses in several biological systems, including cell culture lines and animal, avian and arthropod hosts, (b) the antigenic characteristics of individual viruses, (c) the immunogenic interrelationships between the viruses determining their serological grouping, (d) the role of these viruses, individually and as groups, in the causation of human disease.

Methods Employed:

Viruses belonging to specific serological groups are individually characterized as to optimally susceptible host, in terms of practical virus and antigen production.

Part B included - Yes

Comparative characteristics of the virus placing it within a serological group are determined by *in vitro* complement fixation, neutralization and hemagglutination tests and by *in vivo* neutralization and cross-protection tests. Antigenic analysis with agar-gel and antibody adsorption techniques is attempted. Virus antigens are used to determine the presence of homologous or related antibodies in human sera by standard or specially adapted serological procedures relating the individual viruses and the group to human disease and population experience.

Major Findings:

Each of the 8 viruses was passaged in 5 types of cell culture to establish optimal CPE characteristics; the monkey stable (MS) cell line was found most suitable. Virus strains were standardized concurrently in suckling mice and cell culture with comparable results in the two systems. Plaque studies in cell culture are in progress. Cell culture propagated Bunyamwera virus prototype yielded a hemagglutinin demonstrable by the Takatsy microtitrator technique, but only after special manipulation of the nutrient media. Hemagglutinin can be more easily prepared from suckling mouse brains by sucrose-acetone extraction. Goose erythrocytes are more convenient for both systems and will be used in forthcoming serological studies.

Significance to bio-medical research and the program of the Institute:

Viruses of the Bunyamwera group are widely distributed: isolates from human blood and arthropods have been made in the Old World (East Africa, Czechoslovakia, India) and the New (Trinidad, Brazil, Panama), including the United States (Utah). As yet, human and animal pathogenesis is unknown. We are convinced that early definitive studies with the Bunyamwera viruses and the equally important group C are imperative not only to the discipline of virology but to public health of this hemisphere.

Proposed Course of the Project:

As indicated above, studies of the Bunyamwera viruses will continue; work with group C will be resumed and exploratory investigations with Simbu, California encephalitis and Guama groups will be initiated as time allows.

Serial No. NIAID - 97

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Rosenberger, C. R. and Shaw, C. W. Growth of certain arthropod-borne viruses in hamster kidney tissue culture. Proc. Soc. Exp. Biol. and Med., 106:223-227, 1961.

Serial No. NIAID - 99
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Studies on attraction and feeding behavior of biting and bloodsucking arthropods in relation to preventive measures

Principal Investigator: Paul A. Woke

Other Investigators: None

Cooperating Units: Middle America Research Unit, Panama Canal Zone
Gorgas Memorial Laboratory, Panama, Republic of Panama

Man Years (Calendar year 1961)	
Total:	1 1/2
Professional:	1
Other:	1/2

Project Description:

Objectives:

The objective of the over-all long range program is (1) to learn the causes and mechanisms of attraction and feeding of biting and bloodsucking arthropods (insects, ticks, mites), and (2) on the basis of knowledge of attraction and feeding behavior, to find a naturalistic means for preventing attraction and biting.

Current studies involve attraction behavior of mosquitoes in relation to varied test conditions under development and to differences between individual humans in attractiveness.

Part B included - No

Methods Employed:

Mosquitoes used in the laboratory are *Haemagogus equinus*, *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles quadrimaculatus*. Mosquitoes responding in field tests were various species of *Mansonia*, *Anopheles*, *Psorophora*, *Aedes*, and *Culex*.

On the basis of known behavior characteristics, apparatuses providing varied physical arrangements are conceived, designed, constructed and tested to learn the arrangements that provide the most accurate measures of attraction response. Mosquitoes of varied known ages and conditions are provided with carbohydrate meals and bloodmeals at specified intervals or are subjected to various kinds of environmental preconditioning, following which they are tested for attraction responses. In laboratory experiments on differential attractiveness of humans to mosquitoes, air is circulated in a chamber about the individual or about an appropriate area on the body to pick up emanations. It is conveyed thence to a chamber in which responses of mosquitoes are observed. Accessory arrangements permit variation in rate of airflow and dilution of emanation bearing air. For field experiments a simplified apparatus using natural circulation is available for screening purposes. Man-cages of netting, which allow emanations to escape to the atmosphere, are provided with openings for the arms, so that mosquitoes that alight on a specific area of the arm can be captured, and with means for securing the net to a standard area on the back for the same purpose.

Major Findings:

Progress has been made in determining how the mosquito *Aedes aegypti* responds to a given stimulus differentially according to the varied physical arrangements of the testing apparatus, and more particularly to those conditions that effect the flow and dispersion of emanations. Progress has been made in developing more accurate methods for measuring attraction responses and in developing arthropod cages and manipulative devices that are better adapted to research needs and to efficient and safe handling of arthropods.

Progress has been made in gaining an understanding of the complex manner in which the intensity, periodicity, and selectivity of the feeding responses of *Aedes aegypti* females is correlated with various physical states of the mosquito, particularly with age, kind and quality of prior nourishment, condition of the alimentary canal, state of ovarian development, and atmospheric preconditioning. Cultured mosquitoes *Aedes aegypti*, *Haemagogus equinus*,

Culex quinquefasciatus, and Anopheles quadrimaculatus in studies on feeding behavior and attraction in this laboratory responded differentially to individual humans, some individuals demonstrating much more and some much less attractiveness than others. Indigenous species of wild Anopheles and Mansonia along the Chagres River in the Panama Canal Zone and the Republic of Panama, in the same kinds of studies, responded differentially to individual resident humans of that area, a ratio of 2:1 being not uncommon.

Significance to bio-medical research and the program of the Institute:

Control of certain arthropod-borne virus diseases under certain usual circumstances can be achieved only by reducing the opportunities for the arthropods to serve as vectors. Control of certain other arthropod-borne diseases may be aided by or dependent upon vector control. Mass reduction of vectors or depopulation of affected areas are frequently impractical, even temporarily, especially under emergency conditions such as those of wartime. Personal protection from bites in such cases is essential for control of the disease, and is the only alternative. However, neither physical barriers nor present chemical repellents under usual conditions can reduce biting enough to become practical means for preventing spread of diseases by arthropods. The most certain way by which to find suitable and effective means for preventing biting and bloodsucking is to base the search on a scientific study of the factors involved in biting and bloodsucking. The potential significance of a suitable means by which to reduce bites to a level at which arthropods could no longer effectively transmit diseases cannot be overestimated.

Proposed Course of the Project:

In accordance with a planned background approach to the problems of attraction, biting and bloodsucking, I propose to (a) conduct necessary studies on aerodyanmics and on physiology and behavior of arthropods to provide the information that is essential for the continued development of methods for accurately detecting and measuring responses of arthropods to sources of attraction, (b) to carry out preliminary investigations for the purpose of defining and evaluating various promising approaches by which to learn the causes and mechanisms of attraction, and (c) to conduct studies for the purpose of determining specific conditions and circumstances under which differential attraction occurs.

Serial No. NIAID- 100-A
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

art A:

Project Title: Epidemiological Investigations and Surveillance of Infectious Disease Situations in Panama and Central America

Principal Investigators: Dr. A. Shelekov
Dr. H. K. Beye

Other Investigators: Dr. J. A. Brody, Dr. E. A. Bruckner, Dr. J. V. Ordonez, Dr. R. B. Mackenzie, D. Longfellow, Dr. P. H. Peralta and J. E. Vogel

Cooperating Units: Ministry of Health, Republic of Panama

GOVERNMENT OFFICIALS:
Health Director, Canal Zone
Representative, Zone III, PASB/PAHO/WHO
Chief Surgeons, U.S. Army, Navy and Air Force
U.S. Army Mission Panama

STAFF MEMBERS OF:
Gorgas Memorial Laboratory, R.de P.
Gorgas and Coco Solo Hospitals, C.Z.
C.Z. Military Dispensaries, (U.S. Army, Navy and AF)
Hospital del Nino, R. de P.
Hospital Santo Tomas, R. de P.
Laboratorio Veterinario, R. de P.
Raymond Clinic, R. de P.
Hospital Division, Chiriqui Land Co., R. de P.

art B included Yes

Man Years (calendar year 1961)
Total: 2 2/12
Professional: 10/12
Other: 1 4/12

Project Description:

Objectives:

1) To provide base-line information, liaison, data, and techniques for prompt recognition of changes in morbidity and mortality in Central America;

2) To elucidate the etiology of clinical and epidemic problems in the American tropics;

3) To offer pertinent diagnostic services to the community and public health agencies.

Methods Employed:

Clinical case specimens are submitted by either practicing physicians or public health officals in Panama, the Canal Zone and the neighboring countries, or are collected by MARU medical personnel. In the case of epidemic outbreaks in progress (or when leads of epidemic importance are obtained in the course of testing clinical case specimens) arrangements for field activities are initiated and a separate project is developed if indicated. In the Republic of Panama such arrangements are made through the Director of Gorgas Memorial Laboratory and in the Canal Zone through the Health Director's office. For other Middle America countries two-way communications between MARU and the National Governments are through the Office of the Representative, Zone III of Pan-American Sanitary Bureau, Pan-American Health Organization, WHO.

A mechanism is being developed whereby MARU staff members can maintain close contact with health agencies and physicians working in this area and can secure acute clinical specimens indicative of changes in morbidity. Pertinent data are being collated for Panama and the countries of Central America, which will serve as bases for determining medical situations of epidemiological interest.

Laboratory procedures with specimens from either individual clinical cases or from epidemic outbreaks are generally the same; appropriate cell cultures (as available) and laboratory animals are inoculated for virus isolation, while serological testing of blood specimens for the presence of specific antibodies is performed by standard techniques.

Major Findings:

Several classes of viruses were represented among the 1961 isolates from human specimens. Virus of still another class was isolated from domestic animals during an investigation of an epizootic. From these studies separate projects evolved dealing with Venezuelan equine encephalomyelitis, vesicular stomatitis and measles. Some of the significant findings are mentioned below.

1. Type 1 Poliovirus in Panama. Type 1 poliovirus appears this year to have replaced type 2, which was the type recovered from 12 individuals in the October 1959-March 1960 outbreak of polio on the Isthmus. After the isolation of type 1 poliovirus from 5 patients with clinical polio in October-December 1960, isolations from patients in Panama City or the Canal Zone continued at the rate of approximately one a month to total seven type 1 isolations through August 1961 (with no isolations in April and May). From the Province of Chiriqui (near Costa Rica), specimens from 3 January cases yielded one poliovirus type 1, one type 2 and one which was probably a mixture of both types. Eight of 10 enteroviruses recovered from children surveyed at that time at a Salk polio vaccine clinic in Chiriqui were poliovirus type 1. Specimens from 6 Chiriqui cases in March yielded two type 1 isolates from sisters.

It is of interest that both types 1 and 2 poliovirus were recovered from rectal swab specimens sent from Guatemala in May 1961 from clinical cases of poliomyelitis.

2. Other Enteroviruses. Coxsackie B-3 virus was isolated from 3 adults with pleurodynia in December 1960-January 1961. Several other enterovirus isolates, which were shown not to be poliovirus or Coxsackie viruses B1-5 or A-9, were not further identified.

The ECHO 9 epidemic (reported last year), which began in July 1960, declined by December. In 1961, about 9 months

22

after ECHO 9 infections, serum neutralizing antibodies were found to have persisted (serum titer 1:32) in 8 of 9 children tested.

3. Isolation of Venezuelan Equine Encephalomyelitis (VEE) in Panama. Isolation of VEE virus from specimens obtained from a 14-year-old Panamanian boy, hospitalized at Santo Tomas Hospital in late April 1961 with illness which terminated fatally, marked the first time that this virus had been isolated in Panama. A field and laboratory investigation of VEE was initiated in May as a separate project.

4. Myxoviruses. In March and April 1961, two hemadsorbing agents were recovered which by hemadsorption-inhibition were hemadsorption virus type 1 (para-influenza 3). In March the Division of Quarantine, C. Z., collected specimens from 14 seamen aboard the USS Grant, transiting the Canal following several port calls on the east coast of the U.S. The men had been experiencing an outbreak of URI. No viruses were isolated but results of complement-fixation tests on the single serum specimens supported the clinical impression of an influenza outbreak and indicated that the infections were more likely due to type A although some of the men had also been exposed to influenza type B.

In June and July, there was increased prevalence of respiratory infections - including the influenza syndrome - in the Republic of Panama and the C.Z. Three isolates of influenza type A2 (Asian) were recovered in embryonated eggs. The outbreak, which appeared to be less extensive than the type B influenza epidemic of mid-1959, was not studied further.

5. Vesicular Stomatitis. An epizootic of vesicular stomatitis in dairy cows in Cocle Province, R.P., in late 1960-early 1961 was investigated. When the virus of vesicular stomatitis was isolated, a separate project was developed, since the project on ecology of arthropod-borne viruses in Bocas del Toro Province had earlier suggested the importance of obtaining more information on vesicular stomatitis virus infections.

Significance to Bio-Medical Research and the Program of the Institute:

This project provides epidemic intelligence indispensable to the proper functioning of this unit while offering pertinent

diagnostic services to the medical community. The majority of the other virus projects have been initiated subsequent to careful consideration of leads discovered in the course of willing cooperation with health agencies and individual physicians in Panama and other countries of Middle America.

Proposed Course of the Project:

Investigation of disease outbreaks and of promising individual cases will continue. Projects will evolve from these studies when indicated.

Serial No.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Craighead, J. E., Shelokov, A., Vogel, J. E., Peralta, P. H. An Outbreak of Influenza B in Panama. Amer. J. Trop. Med. & Hyg., 10: 1 (Jan.) 1961.

2. Craighead, J.E., Shelokov, A., Peralta, P.H., Vogel, J.E. Croup Associated Virus Infection in Adults: Report of Two Cases. New Eng. J. Med., 264: 135-137 (Jan. 19), 1961.

3. Craighead, J. E. and Shelokov, A. Encephalomyocarditis Virus Hemagglutination-Inhibition Test Using Antigens Prepared in HeLa Cell Cultures. Proc. Soc. Exp. Biol. and Med. In Press.

Honors and Awards relating to this project:

None

Serial No. NIAID-100-B
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: A Clinical and Virological Study of Oropharyngeal Lesions in Panamanian Children.

Principal Investigator: Dr. J. V. Ordonez

Other Investigators: Dr. S. de Leon (H.del N.), Dr. J. A. Brody and Dr. E. A. Bruckner

Cooperating Units: Hospital del Nino, Panama, R. P.

Man Years (calendar year 1961)

Total:	4/12
Professional:	3/12
Other:	1/12

Project Description:

Objectives:

Clinical and virological evaluation of oropharyngeal lesions in Panamanian children.

Methods Employed:

Swabs were collected in skim milk medium from oropharyngeal lesions in children examined in a pediatric outpatient clinic. It was not feasible to collect paired serum specimens. Test systems for virus isolation were: hamster and monkey kidney cell cultures, and suckling and weanling mice. Tissue-culture neutralization and complement-fixation tests were employed for virus-identification.

Part B included no

Patient Material:

Children with observed oropharyngeal lesions attending the outpatient clinic of Hospital del Nino in Panama.

Major Findings (during the calendar year):

Fifty throat swab specimens were obtained between November 1960 and March 1961 from children ranging in age from 6 months to 11 years (2/3 under 3 yr.). Viral isolates were recovered from 35 patients; there were 32 agents recovered in suckling mice and 34 in hamster kidney HKTC cell cultures. No viruses were recovered from MKTC.

All isolates were identified as herpes simplex virus. HKTC neutralization tests were used to identify 31 HKTC isolates and 28 SM isolates adapted to HKTC. Four SM isolates and 3 mouse-adapted HKTC isolates were identified by the complement-fixation test. Antigens prepared by extraction of infected mouse brain with pH 9 borate-saline (a method suitable for arthropod-borne viruses) proved satisfactory for these tests. Typing serum for both procedures was commercially available.

The characteristics and distribution of the lesions in all cases were compatible with classical herpetic stomatitis and not typical of herpangina with Coxsackie A virus infection.

Significance to Bio-medical Research and the Program of the Institute:

Distressing, often painful lesions of the oropharynx are not uncommon in children seen in the outpatient clinics in Panama. In very small children the condition may lead to severe dehydration when food and water are refused over a prolonged period. Deaths have been reported associated with this clinical picture. The viral etiology of the lesions is important to the understanding of this medical problem.

Proposed course of Project:

The project has been terminated and data are being prepared for publication.

Serial No. NIAID-100-C
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Venezuelan Equine Encephalomyelitis in Panama.

Principal Investigator: Dr. E. A. Bruckner

Other Investigators MARU: Dr. P. H. Peralta, Mr. J. E. Vogel, Miss D. Longfellow, Dr. J. A. Brody and Dr. H. K. Beye

GML: Drs. P. Galindo, C. M. Johnson and M. Grayson

Gorgas Hospital: Drs. J. E. Roberts and H. Mondragon

Santo Tomas Hospital: Drs. G. Omphroy and J. M. Herrera

Cooperating Units: U. S. Army Medical Unit, Ft. Detrick, Md.
Laboratorio Veterinario
Health Director, Canal Zone
Trinidad Regional Virus Laboratory

Man Years (calendar year 1961)

Total	3 3/12
Professional:	1 8/12
Other:	1 7/12

Project Description

Objectives:

1. To investigate - in collaboration with Gorgas Memorial Laboratory (GML) - the epidemiology of Venezuelan equine encephalomyelitis (VEE) virus infection in an area of Panama where the first diagnosed case of VEE

Part B included no

occured (April, 1961).

2. To study virologically and, to a lesser extent, clinically, the original VEE infection and three others which occurred in persons working with the Panamanian strain of VEE virus in the laboratory.

3. To evaluate the responses in laboratory personnel vaccinated with tissue culture-attenuated VEE virus.

Methods Employed:

Collection of arthropods and exposure of sentinel animals were carried out by GML in a one-month field study in Canito, the community from which the fatal case of VEE had come.

In the laboratory investigations, standard methods for the study of arthropod-borne viruses (and particularly methods applicable to group A viruses) have been used. Virus isolation is attempted preferentially in suckling mice (SM); susceptible cell cultures (TC) are used as a second system. For virus titrations and neutralization tests, suckling mice, weanling mice and cell cultures are used, the particular system being chosen according to the test-sensitivity desired. Inactivated antigen for HI and CF tests is prepared by treating VEE-infected mouse brain suspension with beta-propiolactone at 4°C. overnight.

Vaccination of MARU personnel was begun soon after the occurrence of laboratory infections, which were hospitalized at Gorgas Hospital. The vaccine used is an attenuated VEE virus (80th TC passage in Guinea pig heart cells) made available by Drs. Berge and Tigertt of the U.S. Army Medical Unit, Fort Detrick, Maryland. Forty-three persons divided into five groups were vaccinated. Base line information obtained on the vaccinees included histories and physical examinations, WBC and hematocrit. Pre-vaccination serum was obtained. All vaccinees were isolated during the first four post-vaccination days, and were under careful clinical observation.

Major Findings:

One-hundred nine pools of mosquitoes and Phlebotomus sandflies, collected by the GML field team in a period

of one month, failed to yield VEE virus in SM. Two non-VEE viruses, not yet identified, were recovered. No agents were isolated in SM from brain suspensions of chickens which became sick while exposed as sentinels.

CF antibody to VEE virus was encountered in 13%, neutralizing antibody in 16%, and HI antibody in 19% of 117 sera obtained in May from persons living in the general area of Canito. Eight horses from that area were bled in June, and all of these sera contained VEE neutralizing antibody. One of five dog sera from this area was found to have VEE neutralizing and HI antibody. There was also serological evidence of previous experience of humans to Una or a virus related to Una. There was no such evidence of EEE antibody in humans, although there was evidence of previous EEE infection in horses.

Virus was recovered from serum and CNS specimens obtained at autopsy of the Panamanian whose illness terminated fatally. Characterization of the VEE virus strain recovered from serum has been quite limited to date.

VEE virus was recovered from serum and saliva of the first MARU staff member to become ill, and from serum of each of the other two infected workers. All 3 individuals developed high CF titer by 18-23 days after onset, although titer had fallen somewhat within 2 months. The same serum specimens showed neutralizing indices (WM, IP) of the order of 10^7 to 10^8.

Following vaccination serum specimens were obtained daily and viremia was demonstrated within the first three days in only one of the first group of eight vaccinees. Clinical reactions to vaccination in the first 4 days were recorded. They ranged from no symptoms to a 24-hour febrile reaction. From five persons who became ill at 6 to 16 days after vaccination, specimens were collected for virus isolation. By November 1961 four of the five individuals have yielded at least one isolate each of VEE-virus with evidence pointing to its being the attenuated strain.

Serum specimens are being collected at one and three months after vaccination. Of the first 37 of 1-month sera tested, 28 showed HI antibody. Six of the first 13 1-month sera tested for CF antibody had a titer of 1:4 or greater.

Significance to Bio-medical Research and the Program of the Institute:

The virus of VEE had not been isolated in Panama previous to April 1961. Since Panama lies along the fly-way of certain migratory birds, occurrence of VEE virus in Panama may be of importance to other areas of the Americas and specifically to the US where evidence of VEE antibody was recently uncovered in Florida. Epidemiologic as well as ecologic information is important in understanding the local situation and may be applicable in other areas where this virus occurs. Clinical and virological studies on VEE virus infections with the Panamanian strain will add to the general fund of knowledge about a virus which may cause a severe, but probably rarely fatal, systemic infection in man.

Proposed Course of the Project:

Work-up of specimens already collected will continue. No further field work in Canito is anticipated, since the investigation in May 1961 failed to indicate the presence of VEE virus at that time. The antibody levels in serum of MARU personnel will be followed over an extended period in order to learn more about the course of antibody evoked by a virulent strain as well as by an attenuated vaccine strain of VEE virus. In the event of subsequent outbreaks of VEE in Panama, further collaborative studies will be carried out with GML.

Serial No. NIAID-100-D
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Serological Investigation of Measles Virus in Isolated Populations (in Panama)

Principal Investigator: Dr. Jacob A. Brody

Other Investigators: D. Longfellow

Cooperating Units : Ministry of Health, R. P.

Man Years (calendar year 1961)
Total: 5/12
Professional: 3/12
Other: 2/12

Project Description:

Objectives:

The objectives of this project, which was developed following investigation of a measles outbreak in Panama in 1960 are 1) to study measles antibody patterns in isolated communities and 2) to determine which of the common serological techniques - complement-fixation, hemagglutination-inhibition and neutralization - might be most appropriate for field studies on the epidemiology of measles.

Methods Employed:

Forty-nine human serum specimens were obtained toward the end of severe measles in the Cocle mountains in 1960. One hundred sixty-four sera were obtained in the area a year later, fourteen of which were from persons who had been bled previously. Eighty-eight sera were collected in mid-1961 in several Indian villages on the

Part B included no

upper Bayano River, Darien Province, where a measles epidemic had just occurred. Serological tests used are: CF test by standard method; HI test of Rosen using rhesus monkey erythrocytes, and a NT test carried out according to Meyer in Salk monkey heart cell cultures. Sera in HI and NT tests have been tested at dilutions as low as 1:2.

Major Findings (during the calendar year):

Although results are still incomplete, it appears that the HI test is as reliable as the NT test. The correlation of CF with HI and NT data is in progress.

Significance to Bio-medical Research and the Program of the Institute:

It is stated in the literature that measles CF antibody, unlike the CF antibody evoked by many other virus infections persists for a very long time. Study of antibody patterns in isolated communities where measles is not endemic may be expected to show whether this is indeed true. An understanding of CF, HI and NT antibody patterns in isolated populations may be expected to assist in interpretation of results of measles vaccination programs. If vaccine eliminates virus from a community for long periods of time, the serological patterns may more closely resemble these in this study than those reported in endemic areas.

Proposed Course of Project:

Present experiments will be concluded and data carefully evaluated. Whether the project merits continuation will be determined thereafter.

Serial No. NIAID-100-E
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961 .

Part A:

Project Title: Vesicular Stomatitis Virus (VSV) Infections in Panama

Principal Investigator: Dr. J. A. Brody

Other Investigators: Dr. P. H. Peralta, D. Longfellow, Dr. E. A. Bruckner and Capt. G. F. Fischer

Cooperating Units:
Gorgas Memorial Laboratory, R. P.
Laboratorio Veterinario, R. P.
U.S. Army Mission to Panama
Epidemiology Section, Ministry of Health, R. P.
Compania Ganadera de Cocle, R. P.

Man Years (calendar year 1961)

Total:	3/12
Professional:	1/12
Other:	2/12

Project Description:

Objectives:

The objectives of this project were 1) To determine the human infection rates and possible human illness due to the New Jersey type vesicular stomatitis virus, and 2) To investigate the mode of transmission of the virus.

Methods Employed:

A team investigated an epizootic of vesicular stomatitis in cattle from December 1960 through February 1961 in Cocle Province, R. P. One-hundred eight clinical cases

occurred in dairy cows. The Aftosa Center in Rio de Janeiro reported the outbreak due to New Jersey type vesicular stomatitis virus and an agent identified as this virus was subsequently isolated in this laboratory from teat lesion material collected during the outbreak. Virus pools were prepared from infected suckling mouse brain and hamster kidney cell cultures. Human sera obtained from the area of the outbreak and several other areas were tested by neutralization tests in HKTC and by CF tests performed by a standard method.

Major Findings (during the calendar year):

Among persons most in contact with infected cattle, 71% had neutralizing antibody; among those least in contact, 7%. Seven of 92 cattle handlers, but none of the 98 non-cattle handlers, were found to have CF antibody.

In several other widely separated areas of Panama, percentage of human sera with neutralizing antibody to New Jersey type VSV ranged from 0 to 30%.

Data obtained so far suggest that direct contact rather than arthropod vectors may well be more important in transmission of the virus to human beings who have close association with infected domestic animals.

Significance to Bio-medical Research and the Program of the Institute:

Because vesicular stomatitis is endemic and both types of VSV occur, Panama offers a good opportunity to study the viruses which cause a disease of domestic animals, economically important in certain areas of the U.S. and other countries of the western hemisphere, and can infect man as well. Already this project has provided information on human infection rates and a probable mode of transmission in one segment of the population. A better understanding of the ecology of the VS viruses and of their medical importance to man may be anticipated by continuation of this project.

Proposed Course of Project:

Serological testing of selected groups of human beings to discover the populations at risk will be continued, for the purpose of seeking out cases of clinical illness. Long-range plans include study of seasonal patterns of clinical

occurred in dairy cows. The Aftosa Center in Rio de Janeiro reported the outbreak due to New Jersey type vesicular stomatitis virus and an agent identified as this virus was subsequently isolated in this laboratory from teat lesion material collected during the outbreak. Virus pools were prepared from infected suckling mouse brain and hamster kidney cell cultures. Human sera obtained from the area of the outbreak and several other areas were tested by neutralization tests in HKTC and by CF tests performed by a standard method.

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Data obtained so far suggest that direct contact rather than arthropod vectors may well be more important in transmission of the virus to human beings who have close association with infected domestic animals.

Significance to Bio-medical Research and the Program of the Institute:

Because vesicular stomatitis is endemic and both types of VSV occur, Panama offers a good opportunity to study the viruses which cause a disease of domestic animals, economically important in certain areas of the U.S. and other countries of the western hemisphere, and can infect man as well. Already this project has provided information on human infection rates and a probable mode of transmission in one segment of the population. A better understanding of the ecology of the VS viruses and of their medical importance to man may be anticipated by continuation of this project.

Proposed Course of Project:

Serological testing of selected groups of human beings to discover the populations at risk will be continued, for the purpose of seeking out cases of clinical illness. Long-range plans include study of seasonal patterns of clinical

vesicular stomatitis in Panama, investigation of cattle and other vertebrates as hosts or reservoirs of virus, and determination of arthropod vectors in situations in which they are implicated in virus transmission.

Serial No. NIAID-101-A
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Virological Aspects of a Cooperative Investigation on the Ecology of Arthropod-borne Viruses.

Principal Investigators: Dr. P. H. Peralta, Dr. H. K. Beye and Dr. A. Shelokov

Other Investigators GML: Dr. P. Galindo, Dr. E. de Rodaniche and Dr. C. M. Johnson

MARU: D. Longfellow, J. E. Vogel, Dr. E. A. Bruckner and Dr. J. V. Ordonez

Cooperating Units:
Gorgas Memorial Laboratory, R.P.
Hospital Div., Chiriqui Land Co., Almirante, R. de P.
Arthropod-Borne Virus Section, LTV (Bethesda)

Man Years:	(calendar year 1961)
Total:	4
Professional:	1 8/12
Other:	2 4/12

Project Description

Objectives:

1) To isolate and identify arthropod-borne viruses occurring in the tropical rain forest area near Almirante, Bocas del Toro Province, R. de P.;

2) To investigate the role of the virus isolates in human and domestic animal disease;

3) To study the role of arthropod vectors and animal reservoirs in the epidemiology of infections due to the

Part B included no

arthropod borne viruses in the study area and other parts of the Isthmus.

Methods Employed:

Gorgas Memorial Laboratory (GML) provides MARU with arthropods collected in relation to their Bocas del Toro project. These are collected by standard techniques and identified as to genus and, in most cases, species as well. Hamster kidney cell cultures and suckling mice are used for virus isolation. Standard methods for the study of arthropod-borne viruses - including neutralization, complement-fixation and hemagglutination-inhibition techniques - are used for the characterization and identification of isolates and for determination of serum antibodies to the viruses in animals and man. "Immune" ascitic fluids as well as antisera prepared in conventional manner are employed for isolate identification. An experimental method was used for a three-month period for collection of bird blood on filter paper discs for isolation attempts at MARU.

Major Findings:

1. Isolation from Arthropods

The final count of MARU isolates from arthropods collected during the first 12 months of the joint project was 19: 14 from mosquitoes, 5 from *Phlebotomus* sandflies. Suckling mice proved superior to hamster kidney cell cultures as an isolation system. All of the isolates recovered in HKTC showed a cytopathic effect (CPE) in the original passage, although all pools of first-year arthropods were tested by carrying through two passages. In view of this, use of "blind" second passages was discontinued after one year.

By early November 1961 the number of isolates from second-year mosquitoes numbered 19: 14 from mosquitoes and 5 from sandflies (the same number and distribution as for the previous calendar year). The isolation rate from sandflies continued to exceed by far the overall isolation rate from mosquitoes although for certain individual species the rate was high.

2. Isolation from Bird Blood Specimens

Bird blood specimens for virus isolations were collected on absorbent filter paper discs, stored and later eluted

by procedures evolved in the course of another project. Two or three eluates from the same species of bird were combined and inoculated into SM. No viral isolates were recovered in 181 trials which served to screen 488 individual birds for evidence of viremia.

3. Characterization and Identification of Isolates

a. Phlebotomus Isolates. As in the previous year, two groups of agents from Phlebotomus (one "slow" and one "fast") have been isolated. The number of the serologically related Phlebotomus isolates which have been relatively difficult to work with (low titers) stands at 6, with 3 strains from each year of the project. The identity of this serological group has not yet been established, but study of conditions for storage of viral suspensions has led to an improved diluent for these viruses and infectious pools of higher titer. Isolates of Indiana type vesicular stomatitis virus (VSV) from sandflies increased from 2 to 4.

b. Mosquito Isolates. A second-year *Culex nigripalpus* isolate was identified as Indiana type VSV. Identification procedures showed that many of the other mosquito isolates belonged to known serological groups within the arthropod-borne virus class. Six (four being from the first year) are closely related to Una virus of group A. Four others (all second-year isolates) with "group A characteristics" have not been subjected to CFT yet. One strain of Ilheus virus of group B was among the second-year isolates. Members of the Bunyamwera group have been recognized: 1 first-year isolate closely related to Guaroa virus and 4 virus strains (3 of which are from the first year) closely related to Wyeomyia virus. Guama group was found represented by 6 isolates (five being from the first year). The Guama isolates were all derived from *Culex vomerifer* pools; two other *C. vomerifer* isolates have not yet been tested. A few other agents remain unidentified in addition to those mentioned above.

4. Evaluation of "Immune" Ascitic Fluid as a Serologic Reagent.

In the course of serologic work-up of project isolates, mice have been immunized with virus plus an ascites-stimulating adjuvant mixture (after Lieberman et. al. and Kasel et. al.). Ascitic fluid produced has been used in CF tests particularly for relating isolates to one another and to prototype arthropod viruses. Ascitic fluids were found generally to have higher

To further the second and third objectives of the collaborative project, studies will be carried out to delineate the relative importance of some of these arthropod-borne viruses to man and domestic animals in the study area and, when pertinent, in other areas on the Isthmus. Vector and reservoir relationships will be sought for certain of the viruses.

Serial No. NIAID-101-B
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: An Appraisal of Chiggers and other Acarina of the American Tropics for Pathogenic Agents.

Principal Investigator: James M. Brennan

Other Investigators: Conrad E. Yunker

Cooperating Units : Middle America Research Unit, C. Z.
Rocky Mountain Laboratory, Hamilton, Montana
USARCARIB
Gorgas Memorial Laboratory, R. de P.

Man Years: (Calendar year 1961)	
Total:	3 1/2
Professional:	2
Other:	1 1/2

Project Description

Objectives:

A preliminary survey to determine whether chiggers (extremely numerous in species and individuals, and parasitic on all terrestial vertebrates) as well as other parasitic mites are of importance in maintaining and transmitting among wild animals disease potentially communicable to man.

Methods Employed and Major Findings:

This study is undertaken in Panama because of a theory that certain arthropod-borne virus diseases in temperate zones originate in the tropics. Random and intensive sampling of the vertebrate population (with

Part B included no

emphasis on rodents) and subsequent recovery of ectoparasites by various and most effective techniques available have been the means of providing material for laboratory tests.

In the first phase of this study, attempts were made to isolate viruses, using suckling mice and hamster kidney tissue culture, from nearly 500 pools of acarines including 25 species of chiggers, 35 of parasitoid mites, and a few of ticks from over 350 wild vertebrates, mostly mammals. In the first year of this survey, 1665 mammals, 122 birds, and 73 reptiles collected throughout Panama were examined. Mites and ticks were removed from hosts and sorted into species-groups. Each species-group from an individual host was treated as a pool and frozen until processed. Virological techniques employed were adapted from those currently in use at the Middle America Research Unit for the recovery of viruses from arthropods. No evidence of viral activity could be shown in the material under study.

The second phase of the study is being directed toward the assay of similar material for rickettsia. Since the last week in August, live mites and ticks have been sent to the Rocky Mountain Laboratory for test. Total vertebrates examined to date were 450 mammals, 104 birds, and 59 reptiles. Live ectoparasites were removed from 175 of these hosts and sent to the Rocky Mountain Laboratory in 32 shipments totalling 274 pools. Here, an isolation of a rickettsial Q-fever-like agent has been reported, but awaits serologic confirmation. The mite involved is an undescribed species of intradermal chigger from Proechimys, a common spiny rat of wide distribution in the tropics.

Significance to Bio-medical Research and the Program of the Institute, and Proposed Course of the Project:

This particular investigation is relatively new, certainly still in its infancy in the Western Hemisphere, so that it is premature to estimate either its importance to bio-medical research or to predict its course.

Serial No. NIAID-101-C
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Viral "Jungle Fever" in U.S. Military Personnel

Principal Investigator: Dr. J. A. Brody

Other Investigators: D. Longfellow, Dr. J. V. Ordonez, Dr. E. A. Bruckner and Dr. A. Shelokov

Cooperating Units : Lt. Col. J. E. Goldoni, CO,
Jungle Warfare Training Center (JWTC)
U. S. Army
Col. H. E. Leech, MC, Chief Surgeon
USARCARIB

Man Years (calendar year 1961)

Total:	2/12
Professional:	1/12
Other:	1/12

Project Description

Objectives:

1. Isolation of viral agents from the blood of military students participating in a 3-week course at JWTC with illness following known exposure to jungle environment.

2. To determine the rate of human infection with the newly isolated viruses as well as viruses already known to be active on the Isthmus.

Methods Employed:

Specimens are secured by corpsmen from men reporting to the aid station with other than traumatic com-

Part B included no

plaints. These are tested for virus isolation in suckling mice (SM) and hamster kidney cell cultures.

In addition three successive cycles of classes at JWTC were bled before and after the 3-week course. More than 200 paired specimens were collected and tested for antibody to selected viruses known to be present in the area.

Major Findings:

Serum isolate JW-10 reported last year remains unidentified despite serious efforts toward its identification. The virus was shown to be desoxycholate-sensitive, but efforts to identify it by CFT using a battery of arthropod-borne viral antigens and antisera have been unsuccessful. The virus was found to propagate in hamster kidney cell cultures without apparent cytopathic effect. Attempts to adapt it to HeLa cultures are promising.

Six months after his illness (when serum could be obtained) the patient was found to have developed a high level of neutralizing antibody to virus JW-10. Sera from 59 men bled about 3 weeks after beginning the Jungle Warfare training were negative by CFT, and 75 human sera from several areas of Panama showed no neutralizing antibody to this virus.

No other isolates have been recovered during this project.

Significance to Bio-medical Research and the Program of the Institute:

The likelihood that isolates from human blood are of pathogenic significance makes information from this study important in defining not only the local health problems but viral infections of importance to the military training program of the area.

Proposed Course of the Project:

Identity of the single serum isolate will be sought on a continuing basis. In addition, attempted adaptation of the virus to cell cultures will be continued as time allows.

Serial No. NIAID -102-A
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Intestinal Viral Flora in Rural Guatemalan Children - A Survey of Three Villages

Principal Investigators: Dr. P. H. Peralta, Dr. A. Shelokov
Dr. H. K. Beye, Dr. W. Ascoli

Other Investigators MARU: Dr. J. V. Ordonez, Dr. J. A. Brody,
Dr. E. A. Bruckner and J. Vogel
INCAP: Miss V. Pierce, Mr. H. Bruch
Drs. M. Behar, N. Scrimshaw and
J. Gordon

Cooperating Units: Instituto de Nutricion de Centro America y Panama (INCAP), Guatemala

Man Years (calendar year 1961)

Total:	11/12
Professional:	6/12
Other:	5/12

Project Description:

Objectives:

1. To compare the incidence and nature of intestinal viral flora among young children in three carefully controlled villages under constant surveillance.

2. To accumulate information on diarrhea-associated enteroviruses.

Part B included No

Methods Employed:

Rectal swabs are collected by INCAP personnel from all children under 5 years of age in three villages every three months, the villages being visited in rotation at monthly intervals. Data on existence of diarrhea at time of sampling is recorded for each child. Isolation of cytopathogenic viruses in rhesus monkey kidney cell cultures is attempted at MARU. Isolates are to be identified and grouped insofar as possible. At the present time, it does not appear feasible to obtain serum specimens from these children.

Major Findings (during the calendar year):

Results of virus isolation attempts with rectal swab specimens obtained in the first three monthly surveys (February, March, April 1961) are as follows: 83 rectal swabs from Santa Catarina in February gave an isolation rate of 35%, 165 from Santa Cruz in March gave a rate of 23% and 200 from Santa Maria in April, 39%. The swabs from children with diarrhea at time of survey yielded viruses at both higher and lower rates, but numbers of samples were small: Santa Catarina, 53% from 15 swabs; Santa Cruz, only 15% from 26, and Santa Maria, 55% from 33 swabs. The incidence of diarrhea in all villages ranged between 16 and 18%.

Specimens of later surveys are still being tested.

Significance to Bio-medical Research and the Program of the Institute:

This project takes advantage of an unusual opportunity to explore epidemiological aspects of enterovirus infections in relation to nutritional status, as well as status of hygienic and therapeutic measures, in controlled population groups.

From this study much needed background information should contribute to an understanding of the relationships of infection with enteroviruses, nutrition, and possibly manifestations of disease such as diarrhea. Diarrhea and gastroenteritis among children under two years of age is a leading cause of mortality in Guatemala and other Central American countries.

Proposed Course of Project:

It is anticipated that collection and testing of rectal swab specimens will continue for a minimum of 18 months. In this period of time any seasonal patterns might be expected to emerge. Techniques to facilitate enterovirus typing will be explored within this next year. Identification work and correlation of data are expected to extend at least one year beyond the conclusion of specimen-collection.

Serial No. NIAID-103-A
1. Tropical Virology
2. Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Use of Filter Paper Discs for Virus Isolation and Serological Testing

Principal Investigator: Dr. J. A. Brody

Other Investigators: Dr. P. Galindo, GML; and D. Longfellow

Cooperating Units : Gorgas Memorial Laboratory, R. P.
Hospital del Nino, R. P.
Laboratorio Veterinario, R. P.
U.S. Army Mission to Panama

Man Years (calendar year 1961)

Total:	5/12
Professional:	3/12
Other:	2/12

Project Description:

Objectives:

To develop a field tool for virus isolation and for serological testing.

Methods Employed:

Blood specimens included in the year's studies were from human beings, guinea pigs, mice, chickens and wild birds. Human beings were bled by finger puncture, and small animals usually by opening the chest cavity. Blood was absorbed with filter paper discs (Carl Schleichner and Schnell Co., cat. no. 740-E). When virus isolation was planned, discs were kept refrigerated while in the field and subsequently stored at -55°C. When collected for serological studies only, discs were dried

Part B included no

and stored in paper pill-boxes at -20°C. Elution was carried out overnight at 4°C, using 0.6 or 0.75 ml diluent per disc. In comparative studies, specimens of serum diluted as disc elution were likewise allowed to stand at 4°C overnight.

Eluates and diluted sera were tested by standard complement-fixation (CF) and hemagglutination-inhibition (HI) techniques. Kaolin- and acetone-extraction of specimens were compared in a number of HI tests. Screening neutralization (NT) tests were performed in cell cultures: rhesus monkey kidney for enteroviruses and hamster kidney for VS viruses. With disc eluates, the dose of virus was 0.5 logarithm lower than that used against serum diluted 1:8.

Major Findings:

1. Field Trial of the Disc Method for Virus Isolation. Although in 1960 it was found that virus could be recovered from discs which had been soaked with the blood of laboratory animals subsequent to inoculation with EEE, Ilheus, SLE or yellow fever virus, a field trial this year in which 488 bird blood specimens from Bocas del Toro were screened, failed to yield any viral isolates.

2. Application to Neutralization Test. In the screening of blood specimens from 37 children for enterovirus antibodies, the ratio of the number of disc eluates to the number of sera (at 1:8 dilution) which neutralized the test doses of virus was as follows: for poliovirus type 1, 23/25; for type 2, 28/33; type 3, 28/29; for Coxsackie B 2 virus, 21/19 and for ECHO 9 virus, 10/8. In screening of 32 other human blood specimens for antibodies to the two types of vesicular stomatitis virus (VSV), ratios were 26/24 for New Jersey type VSV and 6/7 for Indiana type. The method would appear to be useful for obtaining estimates of the status of populations in regard to neutralizing antibodies.

3. Application to HI Test. HI tests were carried out on sera and disc blood of laboratory animals inoculated with Ilheus virus and of human beings who had been accidentally infected or purposely vaccinated with strains of VEE virus. Only disc blood was available from wild birds. In the succeeding summary, the disc eluate before further dilution is considered as equivalent to serum at a dilution of 1:10.

Kaolin extraction appeared to be acceptable for disc blood as well as serum of the mammalian species. For

Ilheus virus-immunized guinea pigs, disc eluate showed an HI titration endpoint one 2-fold dilution lower than that demonstrated in serum from the same animal, elution and extraction being optimal. For VEE virus-immunized human beings, the HI titer of disc eluate against VEE antigen was at most one 2-fold dilution lower than that of the serum and occasionally was the same. Thus discs promise to be useful for collection of mammalian blood for obtaining rough estimates of HI antibody status of population.

Chicken sera and disc and blood discs required acetone-extraction to minimize non-specific HI, and in general results were not felt to be as reliable as with guinea pigs and man. Nevertheless, in experiments with Ilheus virus-inoculated chickens, when the Ilheus HI titer of serum was greater than 1:40, hemagglutination could usually be detected by disc.

Also, about one-fourth of the eluates from the discs blood of wild birds inhibited hemagglutination by Ilheus antigen, while none of them inhibited VEE hemagglutination.

4. Brief exploration of the possibility of using disc-collected human blood specimens in CF tests indicated that marked hemolysis would present a major difficulty.

Significance to Bio-medical Research and the Program of the Institute:

Filter paper discs are a simple device to facilitate the collection of blood specimens in large numbers, particularly under field conditions. Many discs and disposable stylets for finger-puncture could be readily carried and easily handled in the field. In the laboratory, discs take little storage space. Even newborn infants and very small animals can be bled by this method.

While the use of discs in the isolation of arthropod-borne viruses from blood appears limited, neutralization and HI tests would seem feasible for some viruses at least. Accuracy of results appears to be such that at least valuable background information on the immune status of populations, human or animal, could be obtained.

Proposed Course of the Project:

While many questions on applicability of the disc method for blood collection remain unanswered, this project will be discontinued.

Serial No. NIAID-105-A
1. Tropical Virology
2. Mycotic and Bacterial Diseases Section, MARU
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Studies of Microbial Diseases of Military Importance in Latin-America

Principle Investigator: Major F. H. Diercks, MSC (R&D Command U. S. Army)

Other Investigators : Sgt. Harlan B. Kelley, USAMRU, MARU
Mrs. Martha H. Shacklette, USAMRU, MARU
Capt. Donald G. Moyer, Gorgas Hospital
Dr. Nathan B. Gale, Canal Zone Health Bureau

Cooperating Units : Walter Reed Army Institute of Research
Gorgas Memorial Laboratory
Gorgas and Coco Solo Hospitals, C. Z.
Army, Navy and Air Force Surgeons
U. S. Army Environmental Health Branch
U. S. Army Preventive Medicine Officer
Canal Zone Gov't Health Bureau Div. of Vet. Medicine
Ministry of Health, R. de P.

Man Years (calendar year 1961)

Total:	1/4
Professional:	1/8
Other:	1/8

Project Description:

Objectives:

1. To describe and document microbial diseases and their effects on military and civilian population groups in Latin America.

Part B included no

2. To isolate and identify public health problems affecting military and civilian populations which require additional intensive study and research.

3. To provide research laboratory support for the development of new areas of investigation and the capability for pursuing poorly understood public health problems.

Methods Employed:

In cooperation with military and civilian health authorities and the Epidemiology Section, MARU, efforts will be made to provide for early recognition of an increased prevalence of disease among selected populations. This will include gathering of statistical evidence that an increase in prevalence actually exists. When such evidence is available, then the collection of appropriate specimens will include baseline serological specimens, suitable materials for etiologic studies, and convalescent blood specimens for later study by serologic means. When the basic information has been gathered, a field evaluation of the situation will be made and consultations with other interested laboratories providing base laboratory type support will be sought. In general these studies will avoid long-term projects dealing with chronic disease and/or toxicity or control studies, since these can best be dealt with under more appropriate arrangements with interested agencies. Our prime interest will be in gastro-intestinal disease, acute respiratory disease, or systemic diseases of explosive character and moderate duration.

Major Findings:

During the past year, the participation of the MARU group in operation "Swamp Fox" revealed that among a group of approximately 35 men, who spent 40 days in Panama, there were 3 histoplasmin conversions, 2 men infested with Dermatobium hominis, and three men presenting signs of infestation with Ancylostoma braziliense. In addition, it was established that many men sent to tropical areas for short-term projects bring with them dermatological lesions and mycotic infections which are likely to intensify under tropical conditions. Although this preliminary experience produced no startling or unexpected findings,

it is felt that this approach to the study of infectious disease in the tropics should be pursued further.

Moreover, there are other areas where it is felt that increased surveillance will be fruitful. For example, leptospirosis is known to exist in Central and South America; however to our knowledge there have been few recent isolates of leptospirae from Panama, the Canal Zone, or other countries of Latin America. It is proposed to add to our laboratory armamentarium leptospira culture media, which can be made available to existing hospitals and dispensaries, so that strains may be recovered and specimens obtained which will permit an evaluation of the extent of this disease problem to be made. In a similar fashion, the laboratory capability will be expanded to include at least the preliminary steps in identifying the causative agents of gastro-intestinal outbreaks which may come to the attention of this unit.

Significance to Bio-medical Research and the Program of the Institute:

By expanding the laboratory's capability to participate effectively in investigations into acute infectious disease outbreaks, it is to be hoped that there will result an increased local awareness of infectious disease problems and that more satisfactory methods for prevention and control will ensue. It is to be hoped as the activities of the project expand, our efforts may become truly international and that participation in countries other than Panama and the Canal Zone will result, thus providing an opportunity for expanding our knowledge of the distribution and prevalence of infectious disease agents throughout Latin America.

Proposed Course of the Project:

The primary effort during the past year has been in establishing lines of communication with various military and civilian groups who are willing to participate in these studies. It is hoped that increased surveillance of disease problems among specialized groups of men coming to the Canal for related research and development projects will ensue. For example, it may be possible to strengthen existing liaison with the Jungle Warfare Training Center, Fort Sherman, C.Z., where it will be possible to study individuals reporting into this training center from other Latin American countries. In addition, collaborative studies with other countries will be

sought, and it may be possible to establish liaison which will call to our attention an increase in the prevalence of disease in selected locales, thus providing opportunities for early documentation and definition at the local level. Since this project is in the early months of inception, no definitive report is presented at this time.

Serial No. NIAID-108
1. Tropical Virology
2. Mycotic and Bacterial Diseases
Section, MARU
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Studies of Histoplasmosis on the Isthmus of Panama

Principal Investigators: Maj. R. L. Taylor, MSC and
Maj. F. H. Diercks, MSC (R&D Command, U. S. Army)

Other Investigators : Sgt. Harlan B. Kelley, USAMRU, MARU
Mrs. Martha H. Schacklette, USAMRU, MARU
Dr. Rodolfo Young, Gorgas Hospital
Dr. Nathan B. Gale, Canal Zone Health Bureau

Cooperating Units : Walter Reed Army Institute of Research
Gorgas Memorial Laboratory, R. de P.
Hospital del Nino, R. de P.
Gorgas and Coco Solo Hospitals, C.Z.
Ministry of Health, R. de P.
Canal Zone Health Bureau
Army, Navy and Air Force Surgeons
U.S. Army Environmental Health Branch
U.S. Army Preventive Medicine Officer
Canal Zone Government Health Bureau, Div. of Vet. Med.

Man Years (calendar year 1961)

Total:	4
Professional:	1 1/4
Other:	2 3/4

Part B included yes

Project Description:

Objectives:

1. To determine the public health significance of histoplasmosis in military and civilian population groups in a known endemic region.

2. To define and quantitate certain of the factors associated with the dissemination of *Histoplasma capsulatum* in nature.

3. To provide diagnostic and research laboratory support for the elucidation of the early pathogenesis of clinical histoplasmosis and for the evaluation of theraputic regimens for the treatment of this disease (in cooperation with Gorgas Hospital).

Methods Employed:

In cooperation with military and civilian health authorities obtain and interpret data on sensitivity to histoplasmin in individuals residing in the Canal Zone and the Republic of Panama.

Collect and collate data from known hyperendemic foci to include:

Soil, guano and compost samples for recovery and quantitation of *H. capsulatum*; meteorological data for evaluation of temporal factors influencing dissemination; and recovery attempts from wild animals for definition of their importance as reservoirs of infection.

Major Findings:

During the past year, serologic confirmation of the clinical diagnosis of histoplasmosis has been obtained in 13 patients admitted to the participating hospitals in the Canal Zone and the Republic of Panama. During the year, recovery attempts from sputa (14), urine (34) and other clinical materials (10), were made by inoculation into mice and onto blood-fortified mycosel media without success. Thus it is evident that the approaches to recovery of the organism from clinical materials requires further study.

The histoplasmin skin testing program in the Canal Zone School System has been supplemented by studies done at the Hospital del Nino. Thus, during the past three years, more than 10,000 skin tests have been completed among population groups residing in the Canal Zone and the Republic of Panama. The data obtained indicates that the percentage of reactors increases progressively from the younger to older children and the increase can be related to the length of residence within the area. In addition, it has been possible to obtain skin test data among residents of the San Blas Islands, which indicate that this population group also shows an increasing sensitivity to histoplasmin from the younger to the older residents. The overall incidence of positivity is approximately 50%. When the analysis of the available skin test data is completed, it is felt that the use of this tool for defining the extent of the public health problem presented by histoplasmosis in this region will have been accomplished. Future skin test surveys will be limited to short-term projects in cooperation with the existing health facilities which will broaden our base of understanding of the distribution of this disease.

Laboratory studies of H. capsulatum have included attempts for eliciting antibodies for H. capsulatum in mice. Serum and ascitic fluids from infected mice obtained at varying intervals of time after inoculation were tested by the complement-fixation procedure using histoplasmin and whole yeast phase antigens for evidence of antibodies without success. In addition, attempts were made to elicit an ocular reaction in an immune rabbit by the application of histoplasmin directly into the eye without success. Further, attempts to convert some local strains of H. capsulatum recovered from soil to the yeast phase have been uniformly negative. Thus it is evident that we need to re-direct our efforts in the laboratory to more basic knowledge of the factors influencing the biological behavior of Histoplasma capsulatum.

Epidemiological and ecological studies have continued throughout the year, resulting in the isolation of H. capsulatum from five soil samples obtained from three locales.

The recovery of H. capsulatum from the pooled liver and spleens of bats captured in an artificial harborage at Madden Air Field has clearly opened an additional avenue of investigation into the dissemination of histoplasmosis in this area. From this artificial harborage, the organism has been recovered from compost at the entrance to the building, and from two

pools of liver and spleen suspensions from five individual bats (Chilonycterus rubigonosa fusca). In addition, direct culture of individual livers and spleen pools obtained from 14 Chilonycterus bats captured in the same harborage yielded 9 positive recoveries, thus establishing that this bat species is capable of infection in nature. The role of these bats in the dissemination of Histoplasma capsulatum is yet to be determined and studies are in progress at this artificial harborage and other known harborages in this region. Further studies are in progress at the Madden Tree Site, where the soil is known to be infective and has remained consistently positive during the past three years.

Significance to Bio-medical Research and the Program of the Institute:

In addition to an increased local awareness of the presence of histoplasmosis among the local population groups, this study has produced new leads concerning the epidemiology of this disease in Latin America. The recovery of the organism from soil samples collected in at least six different, widely separated locations on the Isthmus of Panama; the frequent recovery of the organism from known hyperendemic areas such as the Madden Tree Site and the Chilibre caves; and the knowledge that at least one bat species, Chilonycterus rubiginosa fusca, is subject to natural infections, has resulted in a growing chain of evidence which supports the basic concept of the ecology and epidemiology of Histoplasma capsulatum as that of a ubiquitous soil fungus, which is widely distributed in nature. The many factors which may determine the outcome of human contact with the organism under natural circumstances remain to be elucidated. Certainly in the temperate zone, there is a strong association with so called "dusty environments" and resulting clinical disease. The quantitative aspects of dosage of organism, immune status of the individual, the duration of residence in an endemic area, and the relative invasiveness of strains of the organism remain to be investigated. The effect of climatic conditions on known endemic foci have not been defined. The present study offers an opportunity to pursue these investigations in an area of known endemicity.

Proposed Course of the Project:

Primary efforts during the next year will be directed toward quantitative studies of the distribution of Histoplasma capsulatum in nature, and thus to provide the necessary back-

ground knowledge which will allow for development of preventive techniques for protecting individuals obligated to reside or work in known endemic areas.

Serial No. NIAID-108
1. Tropical Virology
2. Mycotic and Bacterial Diseases Section, MARU
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Shacklette, M. H., Diercks, F. H. and Gale, N. B. Recovery of *Histoplasma capsulatum* from Bat Tissues. IN PRINT. *Science*.

Honors and Awards relating to this project:

Serial No. NIAID-109
1. Tropical Virology
2. Mycotic and Bacterial Diseases Section, MARU
3. Panama, Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Studies of Superficial and Deep Mycoses in Panama and Central America

Principal Investigator: Maj. R. L. Taylor, MSC (R&D Command, U. S. Army)

Other Investigators:
Capt. A. W. McFadden, MC, Gorgas Hospital
Mrs. Martha H. Shacklette, USAMRU, MARU
Sgt. Harlan B. Kelley, USAMRU, MARU

Cooperating Units :
Gorgas Hospital, C. Z.
U.S. Military Dispensaries, C. Z.
Veterinary Division, Health Bureau, C. Z.
Raymond Clinic, R. de P.
Santo Tomas Hospital, R. de P.

Man Years (calendar year 1961)

Total:	1/4
Professional:	1/8
Other:	1/8

Project Description:
Objectives:
To establish:

1. The nature of common superficial mycoses in the tropics and the efficacy of newer therapeutic regimens;

2. The extent and types of deep mycoses; and

3. To correlate the abundant airborne mold spores to respiratory allergy.

Part B included yes

Methods Employed:

Standard cultural diagnostic assistance has been made available to local physicians and medical facilities where patients with mycoses are commonly seen. A survey of the airborne molds of Panama was conducted by regularly exposing Petri dishes with medium at six specified locations for one year. Hairbait cultures were utilized for recovery of keratophilic fungi from soil samples which are being processed for Histoplasma capsulatum.

Major Findings:

1. Airborne fungi in Panama. The results of this study have been summarized and are to be reported in Mycopathologia Applicata under the title of "Survey of Airborne Mold Flora in Panama". No further studies are contemplated at this time.

2. Griseofulvin Therapy. Since the evaluation of griseofulvin as a therapeutic agent for chronic myco-dermatologic conditions has been accomplished elsewhere, no further studies are proposed at this time.

3. Therapy of Moniliasis. Since Dr. McFadden has been transferred from Gorgas Hospital, no further studies on this sub-project are projected.

4. Hair-bait Culture of Soils. The results of the hair-bait culture studies are being summarized at present and will be reported in the near future. This sub-project will be discontinued.

5. Epidemiology of Animal and Human Dermatophytosis. No further studies of this sub-project are proposed since the results to date have been essentially unrewarding.

6. Deep Mycoses. The interest in this sub-project will be maintained through studies associated with an alternate project broadening the interest and scope of this section.

Significance to Bio-medical Research and the Program of the Institute:

The exploratory nature of this project has served to delineate several interesting leads of general interest during the past year.

Methods Employed:

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Significance to Bio-medical Research and the Program of the Institute:

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Serial No. NIAID-109
1. Tropical Virology
2. Mycotic and Bacterial Diseases Section, MARU
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Taylor, R. L. and McFadden, A. W. "Survey of Airborne Mold Flora in Panama". IN PRINT. Mycopathologia et Mycologia Applicata.

Calero M., C., Mendez P., G. and Taylor, R. L. "Cervicofacial Actinomycosis: First Report From Panama". The American Journal of Tropical Medicine and Hygiene, X, 877-878, 1961.

Honors and Awards relating to this project:

None

LABORATORY OF GERMFREE ANIMAL RESEARCH

Summary 1

110 - Pathogenesis of Amoebiasis 6
111 - Viral Studies in Germfree Animals 9
112 - Biology of Germfree Animals 11
113 - The Role of the Eosinophilic Leukocyte in the Inflammatory Response to Allergic Reactions and Parasitic Infestation 14
114 - Studies on Bacterial Interactions and Host-Bacteria Relations in the Germfree Animal ... 16
115 - Studies on the Pathogenesis of Infectious and Other Disease Processes in the Germfree Animal 20
116 - Studies on Helminthic Infections in Germfree Hosts 22
117 - Studies on Auto-Immune Diseases and Related Immunologic Processes 24
118 - Tumor Studies in Germfree Mice 28

Laboratory of Germfree Animal Research

National Institute of Allergy and Infectious Diseases

Summary statement of research progress, calendar year 1961

Program developments and trends:

During the year renovation of the South end of the 3rd floor, Building 8, was completed and the research staff of the Laboratory occupied this space during the summer. This move was followed by the renovation of space previously occupied by the staff in the North end of the 3rd floor. With the exception of a special conventional animal room, a cleanup area, and a diet and supply room, the North end is entirely devoted to housing germfree animal units. This represents the final phase of remodeling plans to broaden the scope of research activities of the Laboratory. Four additional germfree units were ordered and should be in the laboratory by the end of December. This will bring our complement to 21 units, including 2 plastics. It is anticipated that we could probably eventually handle 30 units, both steel and plastic, under reasonable conditions.

With regard to personnel, and as we indicated would occur in last year's report, Medical Director Edwin Lerner transferred to our Laboratory from NIAMD in March and S.A. Surgeon (R) Fred Gorstein joined our group on July 1. Dr. Gorstein who has training in immunopathology came to us from N.Y.U. and has worked under Dr. Benacerraf. With the acquisition of these personnel a Section on Experimental Pathology was established. It is planned that this group will be augmented next year by a Research Associate. The Section already has made progress in reviewing, from the pathology standpoint, material from previous germfree research projects. Also, there is underway a fairly extensive collaborative program with the Laboratory of Immunology. From time to time, and on an increasing scale, our pathology staff is called upon for consultation by scientists from other laboratories in our Institute. We have, at the present time, one histo-pathology technician responsible for the many laboratory requirements of these various studies. With the anticipated growth in this area we can expect a need for another technician to handle the more routine aspects of the program. Other than this, it is expected that we should be able to operate at approximately our current level of personnel for the immediate future.

At the risk of sounding repetitious, interest in the use of germfree animals in an increasing variety of research projects continues to grow. More and more, members of our staff are asked to lecture on germfree animal research, techniques, and applications, at scientific sessions and before university staff. At present, in addition to collaborative work with the staff of other laboratories in our own Institute, we are engaged in projects with scientists of the Cancer Institute, CDC, and three universities. We could extend activities with the latter even more so whenever we wish. It is anticipated that this type of cooperation will continue, especially when the cooperating groups have materials, techniques, and tests which will be helpful to us, but which would not be worthwhile for us to attempt to set up. The backlog of projects within our own Institute continues to increase

as our scientists' concern about the 'cost' of the animals is replaced by their obvious value to research.

Extramurally, grants and contracts involving germfree animals and research appear to be continually increasing in number. Our (LGAR and LID) finding that germfree mice have few, if any, viruses is strengthening efforts to apply these techniques to the virus-cancer field. At present, the Laboratory Chief, LGAR, is an advisor on one such extramural project (for NCI), and has been requested to serve in the same capacity in other contracts.

Whether or not we will be able to mount any major projects in this direction, it is felt that more research in basic immunology will gradually trickle into the germfree animal field -- for obvious reasons. The development of simple, non-antigenic diets will soon be a major objective.

Bacterial interactions, such as those relating to genetic transfer of antibiotic resistance, etc., which have been studied so thoroughly *in vitro*, will have, eventually, to be checked out under normal ecological surroundings. The significance and development of L-forms *in the host* will probably receive attention before long, now that this can be done without the confusion of a multiflora. Also, relationships between specific agents and individual bacterial species *in vivo* will probably be the subject of study in the near future. It is my understanding that an international symposium on just this very problem is planned to be held next year

The possibilities offered by germfree, virus-free, or virus-defined animals for serologic, tissue culture and perhaps *pharmacologic* standards will, it is felt, receive increasingly more attention, and has been the subject of discussion among scientists working with germfree animals.

Significant scientific advances:

In the collaborative studies with Dr. Weinstein of LPD our early impression that the sex effect known to occur in certain helminth infections in conventional animals does not occur in germfree animals has been corroborated several times. Whatever the mechanism involved, the lack of a flora reverses the effect normally shown in conventional females. The latter are poor hosts for *Nematospiroides dubius*, for example. We are currently studying mono-infections with individual species of intestinal bacteria in an effort to see if we can pinpoint this interesting relationship. Studies by others working with mono-infected animals have shown that individual species of bacteria can bring about a specific physiologic effect. Among these relationships has been a demonstration of the effect of bacteria on steroid compounds *in vivo*.

An effect of diet on the course, and duration, of certain helminth infections has been observed. This effect appears to be holding up in germfree animals as well as in those reared under conventional circumstances. These infections do not do as well in an animal on a semi-synthetic diet of casein, carbohydrate, vitamins, etc. as they do on the usual Purina-type laboratory animal feed. However, the germfree state appears to compound

the difference. We expect to explore this further during the coming year.

In studies on the occurrence of so-called "natural" antibodies in uninoculated animals, both germfree and conventional, several interesting findings have been made. These studies are being conducted collaboratively with Dr. Cohen of CDC and Dr. Landy of NCI. We have found that mice from a colony which has been reared for at least 7 years free from viable E. coli, S. typhosa, etc., show about the same reactivity to Gram-negative organisms and their products as do conventional animals. No differences were obtained in levels of bactericidal antibody, resistance to endotoxin and phagocytic response. This is of particular interest inasmuch as it had been postulated by others (in theoretical discussions of modes of action of endotoxin) that germfree animals might be considerably more resistant to endotoxin because of their lack of exposure to Gram-negative organisms. It would appear that even though there are no viable, metabolizing, Gram-negative organisms in the animal's intestinal tract, repeated ingestion of small doses of the heat-stabile endotoxin material inevitably present in the diet is sufficient to stimulate the animals.

A similar situation seems to prevail with respect to antibodies or antibody-like activities against Staphylococcus antigen. In a variety of tests involving serum-gel-diffusion, agglutination, and fluorescent antibody techniques, uninoculated animals not harboring any bacteria that could be detected still demonstrated staph antibodies. It was noted that the germfree animals did not develop these "natural" antibodies at as early an age as did the conventional animals. Animals 2-3 months of age were negative, whereas those 7-8 months of age showed considerable activity. Again, one must conclude that prolonged ingestion of small amounts of heat-stabile staphylococcal antigenic material (it has recently been demonstrated that such substances exist) can stimulate the formation of antibodies. These findings point to the source of some of the "natural" antibodies that occur in uninoculated animals. Eventually, we may have to resort to animals raised on soluble diets of small-molecule materials prepared under conditions which (hopefully) would preclude the presence of these, apparently, ubiquitous heat-stabile antigenic materials.

While the study is still in progress, our collaborative work with the NCI on the effect of methylcholanthrene in inducing lung tumors in germfree mice has provided some rather interesting results thus far. The incidence of pulmonary tumors in germfree mice inoculated with MCA subcutaneously as newborns has been 100%, with a mean nodule count of over 100. These findings are as high, and perhaps higher, than the results obtained with such inoculations in the NIH general purpose white Swiss mouse. The response to MCA in "contaminated" mice of the same genetic stock is still underway and optimal comparisons will have to await these results. However, it is already apparent that tumors can be induced in animals protected from contamination about as well as in exposed animals. These findings are of considerable interest in that results thus far indicate that our germfree mice have fewer viruses than the NIH general purpose mouse.

Of further interest is the impression that the incidence of fibrosarcoma and lymphoma is lower in the germfree animals than in the conventional animals. Also, of interest is the fact that deaths due to leukemia have occurred among MCA-inoculated germfree mice.

In our continuing search for evidence of the presence of viruses in our mouse colony, the results continue to be essentially negative for most of the viruses tested. In samplings of well over 125 animals there has been the suspicion of the presence of only one virus in a few instances -- Reo 3. However, according to Drs. Rowe and Hartley of LID who have been performing the tests, their findings are not sufficient evidence to state that the virus is present in the colony, particularly in view of the omnipresence of this virus. Attempts to isolare the agent from bedding, etc., from colony units have not been successful. Among other viruses tested for which negative results were obtained are included mouse adenovirus, poloyma, GD VII, hepatitis and K virus. These studies will continue until we are reasonably sure of the viral state of our colony. However, thus far, it would appear that our germfree mice are free of some of the more common mouse viruses plaguing viral studies in conventional animals.

In collaborative studies with the Laboratory of Immunology, thyroiditis has been produced in some guinea pigs as early as 5 days after immunization. This constitutes the earliest recorded observations of this auto-immune disease in experimental animals. The lesions are found in 100% incidence 16 days after immunization, and at 7 weeks the disease is severe and extensive in all animals. This has persisted as long as 6 months, at the end of which time some decrease in severity has been found. In studies on mechanisms involved in this auto-immune disease, antibody levels, as well as tests for delayed hypersensitivity to thyroid extract, have been followed. Findings thus far suggest that the presence of the disease is correlated with delayed hypersensitivity, but not necessarily with circulating antibodies. Inasmuch as auto-allergy is felt, at present, to be the underlying process in several human diseases, information derived from these studies as experimental models can be of potential importance.

In continued studies aimed at an understanding of the role played by bacteria in aiding Endamoeba histolytica to establish infection and produce lesions in the intestine, efforts were directed at trying to determine whether trauma or damaged mucosa were essential. As we reported previously, it was possible to get some chronic-type lesions in the intestinal wall of germfree guinea pigs with amoebae that were prepared by techniques which apparently resulted in more vigorous organisms than had been used in the past. However, most of the lesions seemed to occur at the site of the inoculation. Recently we have tried inoculations via the terminal ileum whereby the amoebae were delivered into the cecum at some distance away from the puncture wound. Under these conditions the number of germfree animals developing lesions in the cecum was significantly reduced. Thus, at least in the case of the type of lesion obtained in the absence of a flora, trauma or some frank tissue break appear to be necessary for the amoebae to establish infection.

Some further studies on the biology of germfree guinea pigs were conducted. The lack of bacteria seems to have more profound and diverse deleterious effects on guinea pigs than on other laboratory animal species that have been reared. We were able to obtain two litters of second generation germfree animals. Only rarely and recently has this been possible. The growth rate and general physical condition of these young was better than has been experienced with Caesarean-derived animals. The fact that they received maternal milk perhaps may explain their better performance.

Other studies with germfree guinea pigs have also pointed to the inability to define and fulfill requirements supplied by bacteria to this particular species. Germfree animals maintained on the best synthetic diet containing all growth requirements thus far established for guinea pigs did not grow as well nor were they in as good a physical condition as contaminated animals fed the same sterilized diet. This is of particular interest in that other species such as the rat, the mouse, and the chicken seem to do equally as well in the germfree state as in the conventional.

In further studies to ascertain activities of the intestinal flora, it was found that 6-week-old germfree chickens did not contain valeric acid in their intestinal tracts. This substance is a common constituent of gut contents in conventional chickens and, therefore, these findings indicate that it arises from microbial fermentation. Of further interest was the fact that chromatographic analyses of gastro-intestinal material from germfree chickens failed to reveal any fermentation acids in the crop. However, lactic acid was demonstrated to be present throughout the lower GI tract, in amounts which are normally found in conventional birds. This rather unexpected finding in the absence of a demonstrable flora must be explored further.

It is felt that the foregoing review of the past year's findings of even our small group points up the rather fundamental nature of the type of information about host-organism relationships we can expect from studies with germfree animals.

Serial No. NIAID-110
1. Germfree Animal Research
2. Microbiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Pathogenesis of amoebiasis

Principal Investigator: Bruce P. Phillips

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 1-3/4
Professional: 1
Other: 3/4

Project Description:

Objectives:

To investigate factors of potential significance in influencing the etiology, pathogenesis, and pathology of enteric amoebiasis.

Methods Employed:

Germfree guinea pigs, and those with a controlled flora, are inoculated, intracecally, with Endamoeba histolytica by means that will induce various degrees of trauma. The course of the infection is studied, especially from standpoint of gross and microscopic pathology produced.

Major Findings:

Further studies have confirmed the preliminary finding reported last year. Amoebic lesions of a chronic type have been obtained in germfree guinea pigs inoculated intracecally with apparently more vigorous E. histolytica prepared by newer cultivation and harvesting procedures. The lesions, however, were not the ulcerative type that usually develops in the presence of bacterial associates.

When the amoebae were introduced into the cecum via the terminal ileum to avoid the trauma accompanying direct needle puncture of the cecal wall, the number of germfree animals developing lesions was

reduced to a rare occurrence. Thus, at least in the case of the lesions obtained in the germfree animals, trauma or some frank tissue break appears to be a predisposing requirement.

Significance to Bio-Medical Research and the Program of the Institute:

It is known that alleviation of symptoms and healing of lesions often occurs in severe, fulminating amebic enteritis following administration of anti-bacterial but non-amebicidal drugs. Recurrences of the amebic enteritis are almost the rule, however, following the termination of anti-bacterial drug therapy. These germfree animal studies may provide some explanation for the above phenomenon. E. histolytica, although capable of long term survival in bacteria-free tissue, apparently requires active bacterial associates to produce the severe intestinal manifestations of amebic dysentery.

Proposed Course of the Project:

It is anticipated that the current phase of the investigations may be completed by the end of the current calendar year or shortly thereafter. The possible role of Trypanosoma cruzi, the culture associate inoculated with the amoebae, may be investigated by attempting animal-to-animal transfer of amoebae from lesions. It is also felt that our techniques and experience with the strain 200 E. histolytica, may permit undertaking an evaluation of the comparative virulence of other strains of E. histolytica and possibly of E. hartmanni under fairly controlled microbiological conditions. Although the theory of gradient pathogenicity among strains of E. histolytica is rather generally accepted, clearcut experimental evidence is difficult to obtain in the presence of a complex and varying bacterial flora.

Part B Included: Yes

Serial No. NIAID-110

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this Project:

Phillips, Bruce P. and Patricia A. Wolfe: Pneumonic Disease in Germfree Animals. J. Infect. Dis., 108, pp. 12-18, 1961.

Phillips, Bruce P.: Further studies with ameba-trypanosome cultures. Am. J. Trop. Med. Hyg. (In Press)

Honors and Awards relating to this project:

Mr. Phillips was invited to lecture before a medical student seminar at Yale University, New Haven, Connecticut, on November [illegible], 1961.

Serial No. NIAID-111
1. Germfree Animal Research
2. Microbiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Viral studies in germfree animals

Principal Investigator: Vernon J. Fuller

Other Investigators: Wallace P. Rowe, Laboratory of Infectious Diseases
Walter L. Newton

Cooperating Units: NIAID-71-A

Man Years (calendar year 1961):

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

To ascertain what viruses, if any, occur in the LGAR germfree mouse colony. To compare the behavior of viruses in tissue culture systems derived from germfree animals and conventional animals. To ascertain possible interactions between bacteria and viruses in vivo.

Methods Employed:

Standard tissue culture methods, serologic tests, and virologic techniques are employed.

Major Findings:

The germfree mouse colony is being checked serologically for the presence of viral antibodies to polyoma, K virus, mouse adenovirus, Theiler's GD VII virus, and Reo virus. With over 120 animals already tested, no evidence of the presence of these viruses has been obtained with the possible exception of Reo 3. A few positive reactions were obtained; however, it is difficult to conclude much with regard to its endemicity in the mouse colony until further results are available.

Soiled bedding from a germfree mouse colony tank did not contain

virus as indicated by three tissue culture systems and weanling mice studies. Similarly, tumors removed from several germfree mice gave negative results in the two tissue cultures systems employed.

In initial trials mouse hepatitis virus was not demonstrated in the germfree mouse colony through the use of *Eperythrozoon coccoides*. The latter, an apparently harmless parasite which occurs in the blood of mice, exhibits some sort of relationship with MHV. Serial passage of infected spleens and livers almost invariably results in hepatitis in conventional mice after a few passages. In germfree mice with established *E. coccoides* infection, hepatitis has not developed after several passages.

Significance to Bio-Medical Research and the Program of the Institute:

In view of the large number of viruses that have been demonstrated to occur naturally in conventional mice, the value of using animals with no, or even a few known, agents for experimental and diagnostic virologic studies becomes more apparent. The germfree system enables a colony, once virally-defined, to maintain its *status quo*. Furthermore, the demonstration of the presence of virus under such conditions may provide leads in the study of the epidemiology of the infection, particularly with respect to vertical transmission.

Germfree mice or cultures of their tissues may have promise in detecting minute amounts of virus otherwise not detectable in conventional animals or conventional tissue culture. Finally, the study of possible relationships between individual bacterial species and viral agents suspected of being associated in a given disease syndrome can lead to a clarification of the latter.

Proposed Course of the Project:

Attention will continue to be given to the use of indicators and various serologic tests to examine the germfree mouse colony for other possible latent viruses. Comparison of the susceptibility of germfree- and conventional-animal derived tissue culture systems to certain viruses will be resumed.

Part B Included: No

Serial No. NIAID-112
1. Germfree Animal Research
2. Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Biology of germfree animals

Principal Investigator: Richard E. Horton

Other Investigators: Walter L. Newton
Georg F. Springer, University of Pennsylvania
W. E. C. Moore, Virginia Agriculture Experiment Station

Cooperating Units: DRS-6.4

Man Years (calendar year 1961):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

To compare various biologic, serologic and physiologic characteristics of germfree animals with those of conventional animals. Where differences are encountered, to ascertain whether particular bacterial species are involved, and in what manner.

Methods Employed:

Standard laboratory techniques normally used in comparative studies of serology, hematology, growth, fecundity, etc., are employed.

Major Findings:

In further efforts to promote reproduction in Caesarean-born germfree guinea pigs, the amounts of protein and certain vitamins were increased, together with a decrease in the amount of fat. Two litters of second generation germfree guinea pigs were born, and some animals were maintained for 3 months before they became accidentally contaminated. The growth rate and general physical condition was better in these animals than has been experienced with

Caesarean-derived animals. The fact that these second generation animals received maternal milk may explain their better performance.

In studies on the origin of anti-human R agglutinins, relatively high doses of purified E. coli 086 lipopolysaccharide injected intravenously gave a strongly positive Coomb's test in conventional animals. Similar injections of E. coli 086-monocontaminated chicks resulted in a negative Coomb's test. Apparently the conventional chicks contain incomplete antibodies reacting with their own red cells. In contrast to germfree chicken blood which contains neither group B-like nor group A-like antigen, the blood of 5-month-old germfree mice contains these antigens. The latter are known to exist in conventional mice and conventional chicks.

In studies to evaluate responses to synthetic antigens, it was observed that germfree chicks developed antibodies to a synthetic antigen coupled to a protein (L-fucose phenyl-azo horse serum albumin), almost as well as conventional chicks. However, neither germfree nor conventional guinea pigs exhibited this response.

It has been demonstrated that 6-weeks-old germfree chicks do not contain valeric acid in their intestinal tracts. Valeric acid is a common constituent of the gut contents in conventional chicks, and these findings suggest that it arises from microbial fermentation.

Chromatographic analysis of the gastro-intestinal material from the germfree chicks revealed the absence of all fermentation acids in the crop. However, lactic acid was present throughout the lower intestinal tract in amounts which are normally found in conventional birds. This unexpected amount of lactic acid in the intestinal contents of chickens without a demonstrable flora needs further exploration because of its interesting implications.

Significance to Bio-Medical Research and the Program of the Institute:

Studies of this nature can provide fundamental information on the ways in which the "normal" flora may affect various physiologic and biological characteristics of the host.

Proposed Course of the Project:

Studies will continue along essentially the same lines. Some species variation with regard to the effects of the intestinal flora has been encountered. Thus, germfree guinea pigs die rather quickly when brought to the "outside". Germfree mice generally do not. Efforts will be made to ascertain possible reasons for this via previous immunization, mono-infections, etc.

Part B Included: Yes

Serial No. NIAID-113

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Horton, Richard E. and John L. S. Hickey: Irradiated diets for rearing germfree guinea pigs. Proc. Animal Care Panel, 11:(2), pp. 99-106, 1961.

Newton, Walter L. and William B. DeWitt: Nutrition and serum protein levels in germfree guinea pigs. J. Nutr. 75:(2), pp. 145-151, 1961.

Honors and Awards relating to this project: None

Serial No. NIAID-113
1. Germfree Animal Research
2. Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: The role of the eosinophilic leukocyte in the inflammatory response to allergic reactions and parasitic infestation

Principal Investigator: Fred Gorstein

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives:

To ascertain whether the eosinophil is an immunologically competent cell. To determine the composition of the eosinophil granule and its alteration in the course of various tissue reactions.

Methods Employed:

After the basic method of Burner et al, eosinophils are explanted on chick allantoic membranes to determine whether the cell is capable of producing a "graft against host" reaction. In inbred guinea pigs with thyroiditis, eosinophilic leukocyte-rich exudates are utilized in an attempt to transfer the lesion to normal recipients. Guinea pigs subjected to a variety of antigens, antigen-antibody complexes or parasitic infestations are given horse serum to elicit an eosinophil leukocyte-rich peritoneal exudate which is further refined to obtain virtually pure cell suspensions. Cells are disrupted by ultrasonication and the granules are separated by differential centrifugation. Determinations of the activity of various enzymes will be made.

Major Findings:

This project has just been initiated. Eosinophil-rich exudates have been produced in guinea pigs and mice. Techniques are being studied to further purify these exudates. Thyroiditis has been produced by active immunization with thyroid extract in guinea pigs.

Significance to Bio-Medical Research and the Program of the Institute:

To date the role of the eosinophil remains an enigma. Its invariable presence in allergic and parasitic inflammatory reactions leads one to explore the mechanism of [illegible] the role of the cell at these sites. While it has been postulated that the cell is immunologically competent, no conclusive evidence is available.

Information as to the composition of the eosinophilic granules (specifically with respect to alterations in [illegible]) should shed some light as to the role of the cell in the inflammatory reaction.

Proposed Course of the Project:

The immunologic studies of the eosinophil will be expanded by analyzing the immunoelectrophoretic pattern of the cell with anti-rabbit onto guinea pig eosinophil serum.

The enzyme constituents of the eosinophil will be studied during the course of a variety of allergic and infectious disease inflaminatory reactions.

Part B Included: No

Serial No. NIAID-115
1. Germfree Animal Research
2. Microbiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Studies on bacterial interactions and host-bacteria relations in the germfree animal

Principal Investigator: Norman S. Ikari

Other Investigators: Walter L. Newton
Maurice Landy, National Cancer Institute

Cooperating Units: CDC, Atlanta, Georgia

Man Years (calendar year 1961):

Total:	2-1/4
Professional:	1-1/4
Other:	1

Project Description:

Objectives:

To determine whether certain bacterial phenomena observed *in vitro*, e.g. genetic transfer of antibiotic resistance between strains of bacteria, occur within the intestine of the living mammalian host. To evaluate host responses, particularly immunologic, to individual bacterial species in the absence of other microorganisms.

Methods Employed:

Escherichia coli K 12 Row (streptomycin-resistant, colicine-sensitive) is established in the germfree mouse intestinal tract. Later, Paracolon CA62 (streptomycin-sensitive, colicine-positive (col +)) is fed by mouth tube, and fecal pellets are collected at intervals thereafter. Saline dilutions of these pellets are assayed for col + colonies by plating onto streptomycin agar plates.

Sera of germfree and variously monoinfected mice are compared for levels of antibody activity towards specific organisms and soluble antigens, using the serum-gel diffusion, agglutination and fluorescent antibody techniques.

Major Findings:

A previously non-colicinogenic species of E. coli produced colicine after association in vivo with a [illegible] species which was a colicine-producer.

Antibodies to staphylococcal antigens were demonstrated to occur in 6- to 8-month-old mice in which no viable bacteria could be detected. These antibodies were absent in 2-month-old germfree mice, whereas they were present in young conventional mice of the same age and strain, and fed the same sterilized diet. Apparently, over a period of time the germfree animals ingest sufficient heat-stabile staphylococcal antigens from the diet to stimulate the production of antibodies.

The same situation was found to exist with respect to antibodies to gram-negative organisms. Germfree mice had about the same levels of bactericidal antibody to Salmonella typhosa and E. coli as conventional mice. Likewise the uninfected animals showed about the same levels of response to the lethal effects of endotoxin as did conventional animals. Previous speculation had suggested that the germfree animals, because they have not been exposed (and perhaps sensitized) to Gram negative organisms might be more resistant to endotoxin. As with the staphylococci, it may be that sufficient heat-stabile antigenic material is present in the sterilized diet to stimulate antibody production in the uninfected animals.

Significance to Bio-Medical Research and the Program of the Institute:

Much of our information on bacterial interactions, genetic changes, resistance development, etc., has, of course, been acquired from in vitro studies. Now that animals are available without additional microorganisms to complicate relationships, it seems logical to review some of the latter under more natural ecologic conditions. The host may very well influence the course of some of these phenomena.

Information as to the origin of, and mechanisms leading to, so-called "natural" or "normal" antibodies in uninoculated animals can contribute to the overall understanding of immunologic responses.

Proposed Course of the Project:

Additional genetic recombination systems and "markers" will be studied in vivo, especially those involved with virulence and antibiotic resistance. It is also planned to collaborate with LBD in a study of the behavior of L-forms in germfree animals.

Efforts will be made to ascertain which materials are stimulating

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Fite, George L. and Walter L. Newton: Leprosy inoculations of germ-free guinea-pigs. Internat. J. Leprosy, 29:(2), pp. 189-190, 1961.

Landy, Maurice, James L. Whitby, J. Gabriel Michael, Mark W. Woods, and Walter L. Newton: The effect of bacterial endotoxin in germfree mice. Soc. Proc. Exp. Biol. & Med. (In Press)

Honors and Awards relating to this project:

Dr. Walter L. Newton was invited to give the guest lecture at the annual meeting of the North Carolina Bacteriological Society at the Bowman Gray School of Medicine.

Serial No. NIAID-115
1. Germfree Animal Research
2. Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Studies on the pathogenesis of infectious and other disease processes in the germfree animal

Principal Investigator: Edwin M. Lerner II

Other Investigators: Fred Gorstein

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1-1/4
Professional:	1/2
Other:	3/4

Project Description:

Objectives:

To undertake a comprehensive and comparative study of the reaction patterns in the germfree and conventional animal, with particular reference to morphological changes, specific immune reactions, and non-specific host defense mechanisms in response to infectious, immunogenic, and other stimuli. To ascertain causes of death in animals without demonstrable infection.

Methods Employed:

General morphologic data are compiled and catalogued by the routine autopsy of all germfree and conventional animals utilized in the total program of the Laboratory of Germfree Animal Research. Complete gross and histologic examinations are performed, and when indicated for particular experiments or groups of animals specialized histochemical and other techniques are added.

In addition, germfree and conventional animals are exposed to various specific pathogens or allergens by appropriate routes. These animals are followed for clinical disease, studied bacteriologically and immunologically, and subsequently by a complete morphologic examination. Tests of non-specific defense reactions will include carbon clearances for reticulo-endothelial system function and bactericidin titres.

Major Findings:

This project has just recently been initiated. The general range of pathological findings in the germfree host thus far has been essentially similar to that in conventional animals. Specific lesions observed in a considerable number of older (12-24-months) germfree mice include: development of extensive carcinomata and adenomata of the lung, reticulum-cell sarcoma, bilateral enlargement and hemorrhagic lesions of the ovaries, and urethral calculi with secondary bladder enlargement. All of these lesions have been observed in conventional animals. It is too soon to tell whether quantitative differences occur, or whether the presence of a flora influences the incidence or severity of these lesions.

A study of the morphology of N. dubius infection in germfree and conventional hosts has, to date, failed to show any morphologic difference in the intestinal lesion.

Significance to Bio-Medical Research and the Program of the Institute:

The influence of previous infection and/or immunogenic experience on experiments involving the infection or immunization of animals has been known to exercise varying effects. It would be of great value to define infectious or immune processes in experimental animals which have had little or no prior natural or accidental infection or immunization. Thus, the actual host defense response, both specific and non-specific, and the pathogenesis of a given infectious process may conceivably be defined in a system which excludes the influence of these possible variables.

Proposed Course of the Project:

Germfree and conventional animals used in infection and immunization experiments will be studied morphologically in detail. In particular experiments, bacteriological and immunological tests will be performed as indicated, in an attempt to correlate morphologic changes with these, and to determine any differences in the various responses between the germfree and conventional animals.

Part B Included: No

Serial No. NIAID-116
1. Germfree Animal Research
2. Microbiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Studies on helminthic infections in germfree hosts

Principal Investigator: Walter L. Newton

Other Investigators: Paul P. Weinstein, Laboratory of Parasitic Diseases
William B. DeWitt, Laboratory of Parasitic Diseases

Cooperating Units: NIAID-122-F

Man Years (calendar year 1961):
Total: 1-1/2
Professional: 1/2
Other: 1

Project Description:

Objectives:

To ascertain what effect the bacteria and other organisms normally present in the conventional animal have upon the course of helminth infections, and upon the host's response to these parasites.

Methods Employed:

Germfree animals are fed or inoculated with sterilized eggs, or axenically-reared infective larvae, of various parasitic helminths. The development of these parasites is followed by established procedures, and lungs, intestine, and other appropriate tissues are examined histologically for the study of host response. The findings are compared with those obtained in conventional controls.

Major Findings:

Further studies have established with certainty the previous impressions that the sex effect noted in conventional mice infected with Nematospiroides dubius does not occur in germfree mice. Whereas female conventional mice have 1/3-1/2 the worm burden of the males following the same inoculum, female germfree mice do not show this reduction. We are now in the process of checking this phenomenon in

monocontaminated mice.

As reported previously, this parasite (which is maintained in conventional stock) develops about as well in the germfree host as in the conventional animal. We have been able to complete three cycles in a sterile environment, that is, pass the parasite only through the germfree animal. However, there has been some evidence that the infection gradually becomes less vigorous than when it is passed in conventional animals. If this tendency continues it could indicate that some subtle deficiencies were accumulating in the absence of an intestinal flora.

Significance to Bio-Medical Research and the Program of the Institute:

An intestinal parasite-host system free from complicating bacteria can provide rather direct information in the areas of nutrition and parasitism, and on the physiology of parasites under more normal conditions than *in vitro* cultivation. Opportunity is provided for the study of eggs and larvae in fresh (non-sterilized) feces without bacteria, and perhaps to ascertain factors contributed by the latter toward their proper development. If we can shed light on the sex phenomenon, interesting and potentially far-reaching relationships may be uncovered.

Proposed Course of the Project:

Efforts will be made, through the use of animals infected with single species of bacteria, to elucidate the host sex effect. Attempts will be made to ascertain whether the intestinal flora are involved in certain parasite-diet relationships noted in conventional mice.

Part B Included: No

Serial No. NIAID-157
1. Comparative Animal Research
2. Experimental Pathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Studies on auto-immune diseases and related immunologic processes

Principal Investigator: Edwin H. Lerner II

Other Investigators: Fred Gorstein
Philip R. B. McMaster, Laboratory of Immunology
Sanford H. Stone, Laboratory of Immunology
Howard [illegible], Laboratory of Immunology

Cooperating Units: NIAID-150

Man Years (calendar year 1961):
Total: 1
Professional: 1/4
Other: 3/4

Project Description:

Objectives:

To produce experimental allergic thyroiditis and other auto-immune diseases in inbred animals, and to study the developmental pathology of the disease and its relationship to the immune response.

Methods Employed:

Inbred guinea pigs are immunized with antigens prepared from guinea pig thyroid in Freund's adjuvants, and are killed at intervals to chart the development of thyroiditis. Serum antibodies are determined by a modified bentonite flocculation test or the tanned red cell agglutination technique, and hypersensitivity is determined by skin testing with thyroid antigens.

Similar immunization with [illegible] is being carried out.

Major Findings:

Thyroiditis has been produced in [illegible] as early as 5 days after immunization, constituting the earliest recorded

observation of this auto-immune disease in experimental animals. The lesions are found in 100% incidence 5 days after immunization, and at 7 weeks the disease is severe and [illegible] in all animals. This has persisted as long as 6 months, at which period some decrease in severity has been found.

Delayed hypersensitivity to thyroid extract, as determined by skin test, has been noted in most animals at 5 days after immunization and has been consistently associated with the finding of thyroiditis, at both the 5-day and the 7-week stage. Some decrease in skin test hypersensitivity has been found at 6 months.

The appearance of circulating antibody to thyroid has not been consistently correlated to thyroiditis. Antibody was absent at 5 days, although thyroiditis and delayed hypersensitivity were found. Antibody was present at high levels at 7 weeks, and paralleled the severity of lesions. Antibody began to disappear at 3-1/2 months, although thyroiditis was still [illegible] present and severe.

Thus, the presence of this auto-immune [illegible] to correlate with the finding of delayed hypersensitivity, but not with circulating antibody.

Approximately 10-15% of inbred mice immunized with mouse thyroid extract develop definite though minimal lesions of the thyroid within 3 weeks.

Significance to Bio-Medical Research and the Program of the Institute:

These findings help to clarify understanding of an experimental auto-immune disease which is an excellent model for elucidating the mechanisms of its human counterpart, Hashimoto's thyroiditis. The latter is believed to be the result of an auto-immune process. The variations in circulating antibody during the course of the disease in experimental animals help to explain the widely divergent findings of antibody levels in human thyroiditis. If delayed hypersensitivity *per se* is responsible for this disease, it should be possible to demonstrate this experimentally, and to determine whether or not circulating antibody can be totally excluded. This model is more clearly defined than are the other experimental auto-immune diseases such as encephalomyelitis or aspermatogenesis. Since auto-allergy is felt at present to be the underlying process in several human diseases, any information derived from these experimental models is of great potential importance.

Proposed Course of the Project:

The development and regression of [illegible] thyroid lesions in guinea pigs will be followed as long as [illegible]

or lesions persist. Similarly, the course of hypersensitivity and of circulating antibody will be charted. Efforts are in progress to transfer the delayed hypersensitivity, and consequently the thyroiditis, by means of lymph node transfer from actively immunized animals, taking advantage of the inbred Strain 13 guinea pigs. Attempts are also being made to transfer this immunity with cell-free extracts of lymph nodes, in collaboration with Dr. John Turk, a visiting scientist from the National Institute of Medical Research, England.

Attempts are also being made to produce experimental allergic thyroiditis in inbred mice, and to study this in fashion similar to the guinea pig disease. In mice, efforts will be made to transfer immunity (and the disease) by the use of thoracic duct lymphocytes and by cell-free lymph, and to localize the active cells in recipient animals by radioautography.

Part B Included: Yes

Serial No. NIAID-117

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

McMaster, Philip R. B., Edwin M. Lerner 2nd, and Eurmal D. Exum: The relationship of delayed hypersensitivity and circulating antibody to experimental allergic thyroiditis in inbred guinea pigs. J. Exptl. Med., 113:(4), pp. 611-624, April 1961.

McMaster, Philip R. B., Edwin M. Lerner II, and Eurmal D. Exum: Experimental allergic thyroiditis in inbred guinea pigs: Some immunologic and pathologic aspects. Fed. Proc., 20:(1) (Part I), March 1961.

Honors and Awards relating to this project:

Dr. Lerner was appointed official delegate of the American Association for the Advancement of Science at the inauguration of Randle Elliott as President of Hood College, Frederick, Maryland, on October 14, 1961.

Serial No. NIAID-118
1. Germfree Animal Research
2. Microbiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Tumor studies in germfree mice

Principal Investigator: Walter L. Newton

Other Investigators: Margaret Kelly, National Cancer Institute
Roger O'Gara, National Cancer Institute

Cooperating Units: NCI-1-3505

Man Years (calendar year 1961):

Total:	3/4
Professional:	1/4
Other:	1/2

Project Description:

Objectives:

To study the development and comparative incidence of spontaneous and induced tumors in germfree and conventional mice of the same strain.

Methods Employed:

Uninoculated mice that die or are moribund are examined both grossly and histopathologically for the presence of tumors, particularly lung tumors. Day-old mice are inoculated subcutaneously with graded doses of 3-methylcholanthrene in olive oil.

Major Findings:

The study is still in progress, and final evaluation of results cannot be made at this time. To date, however, the incidence of pulmonary tumors in mice killed between 16-24 weeks after inoculation with either 0.1 mg or 0.3 mg methylcholanthrene subcutaneously as newborns has been 100%, with a mean nodule count of 100 or more. In a comparison of the susceptibility of these white Swiss mice with that of the NIH General Purpose mouse, the incidence and mean nodule count of lung tumors appears to be higher in the germfree animal, and the incidence of fibro-sarcoma and lymphoma is lower. The

response to MCA in conventional mice of the same genetic stock is under study and optimal comparisons will have to await these results. However, it is already apparent that tumors can be induced in germfree white Swiss mice at about the same incidence level as in conventional "contaminated" mice.

Findings thus far are of considerable interest in that it is likely that the germfree mice have fewer viruses than the conventional general purpose animal exposed to the outside environment. As mentioned in our project report NIAID-111, of 6 mouse viruses tested for so far, there is suspicion of the presence of only one, Reo-3.

Significance to Bio-Medical Research and the Program of the Institute:

There is a well-known and increasing interest in the possible role of viruses in the development of certain tumors. Perhaps chemical carcinogens that induce tumors may in some way activate latent viruses.

Studies conducted in animals demonstrated to be free of, or containing a limited number of, known viruses and other organisms can lead to the establishment of more definite relationships than has been possible with many of the apparently virus-laden mice used today.

Proposed Course of the Project:

This project will continue along present lines. Additional carcinogenic agents will probably be tried. Attempts will be made to isolate cell-free fractions from tumors in germfree mice to determine if such fractions can induce tumors in other mice.

Part B Included: No

LABORATORY OF PARASITIC DISEASES

Summary 1

120 - Administration and Research Planning and Coordination 8
121-A - Host Parasite Relations in Worm Infections in Laboratory Animals 9
121-C - The Biology and Control of the Snail Intermediate Hosts of the Schistosomes ... 13
121-D - Investigation of Intermediate Hosts and Vectors of Human Diseases Caused by Worms 16
122-A - The *in Vitro* Culture of Parasitic Helminths 19
122-B - The Development of Helminths in Germfree Animals 23
122-C - The Role of Helminths in the Etiology of Eosinophilic Meningitis and Meningo-encephalitis 25
122-D - Effects of Nutrition on Chemotherapy of Parasitic Diseases 28
122-E - Effects of Improving the Nutrition of Malnourished People Infected with *Schistosoma Mansoni* 31
122-F - Studies on the Influence of Dietary Factors on Experimental Parasitic Infections 35
122-G - Studies on *Entamoeba Histolytica* and Other Parasitic Protozoa 38
122-H - Biochemistry of Parasitic Protozoa 42
123-A - Fundamental Physiological and Biochemical Studies on Parasites, Intermediate Hosts, and Parasitized Animals 45
123-B - Pathological Physiology and Histochemistry of Parasitic Diseases 49
123-C - Biochemical Mechanisms of Energy Metabolism in Normal and Parasitized Animals 52
123-D - Biology of Trypanosomes 56
124-A - Effect of Radiation on the Snail Hosts of *Schistosoma Mansoni* and on Schistosome Cercariae .... [illegible]
124-B - Pathology of Acute and Chronic *Schistosoma Mansoni* Infection in the Mouse, Hamster, and Multimammate Rat (*Mastomys*) .. [illegible]
124-C - Effect of Gelatin on the Rate of Clearance of Colloids from the Blood 64
125-A - Studies on Toxoplasmosis .... [illegible]
125-B - Serology of Toxoplasmosis .... [illegible]

125-C - Trichomoniasis 73
125-D - The Pathophysiology of Schistosoma Mansoni Infection in the Experimental Animal 76
125-E - Ammonia Metabolism and Toxicity in Relation to Liver Disease 79

Laboratory of Parasitic Diseases
National Institute of Allergy and Infectious Diseases

Summary statement of research activities, calendar year 1961

Program development and trends:

Reorganization of the Laboratory of Parasitic Diseases became effective on January 9 of this year. The Laboratory now contains the following Sections: 1) Physiology and Biochemistry, 2) Host-Parasite Relationships, 3) Ecology, Biology, and Field Studies, and 4) Clinical Parasitology. These changes represent shifts away from the classical taxonomic basis for section organization to a more functional and less restrictive orientation. The new Sections are organized to group together investigators with similar problems regardless of the taxonomic group of organisms being studied. The first two Sections are designed to encompass the study of basic parasitological problems. The other two, "Ecology, Biology, and Field Studies" and "Clinical Parasitology", recognize our responsibility to provide knowledge and competence regarding the epidemiology and control of parasitic diseases and in the study of their clinical and pathologic aspects. Both of these Sections are expected to conduct basic as well as applied research.

The following changes in permanent staff occurred: Dr. Jacobs returned from New Zealand in May to resume his duties as Chief of the Laboratory and Acting Head of the Section on Clinical Parasitology. Miss Lucy V. Reardon retired in July, and her position was filled by transfer of Dr. Morris Goldman from the Communicable Disease Center, Atlanta. Dr. Goldman reported for duty on October 31. Arrangements have been made, also, for the transfer of Dr. Charles Richards of the CDC field station in Puerto Rico to this Laboratory to fill a long-standing vacancy in the Ecology, Biology, and Field Studies Section; this transfer will be made in January of the coming year.

Two guest workers, Mr. Ian Sommerville and Dr. Iain R. Bowman, departed in April and September after 1 1/2 and 2 years of work with Dr. Weinstein and Dr. von Brand respectively. Two new guest workers are now engaged in collaborative projects with permanent staff members. They are Dr. Alina Szumlewicz, of Brazil, who arrived in July and is working with Dr. Berry on the effects of irradiation on snails and cercariae; and Dr. Akio Kobayashi, who arrived in October and is working with Dr. Jacobs on the effects of irradiation on *Toxoplasma*.

Some project changes have developed along with shifts in personnel. Work on trichomoniasis from the standpoint of pathogenesis of strains, antibody-formation, and chemotherapy with purportedly active drugs, etc., has been de-emphasized since Miss Reardon's retirement. Dr. Goldman's efforts, instead, will be devoted to the application of immuno-fluorescence techniques to the study of strain differences within the species *Entamoeba histolytica*. We have extremely little basic information on which to characterize strains of parasites. They are mostly designated by origin and described on the basis of

differences in virulence, culture, etc. The immuno-fluorescence techniques may be useful here, and work on freezing of parasites will allow the preservation of strain characteristics. If circumstances are propitious, an effort will be made to extend Dr. Goldman's techniques to studies on helminthic antigens as well.

Our emphasis is on basic work, with the general philosophy that field studies should represent the application or exploitation of special techniques or concepts developed at the bench. The program of the Laboratory is well diversified, considering the size of the staff, and the competencies of the various staff members cover a large part of the field of human parasitology. Efforts are being made continually to broaden our scope. A case in point is the work on the cultivation of filariid parasites that has been initiated. The program on filariasis may be extended somewhat when Dr. [illegible] joins the staff depending on the availability of material and the development of an insectary. An insectary is badly needed already for the culture work and life cycle studies on filariids. Thus, we will be focusing attention on the helminthiasis which is second only to schistosomiasis as a global health problem. The emphasis on filariasis will continue to be on basic problems until such time as there develop leads that must be exploited in the field. Another extension of work on helminths is the study of *Angiostrongylus cantonensis* in slugs, rats, and monkeys in the laboratory.

The Puerto Rico project conducted by Dr. DeWitt on the relation of nutrition to schistosomiasis, is almost completed. Of a series of projects proposed for the use of PL 480 funds in various countries, only one to be performed by Dr. Warren in Brazil is about to be set up. This project is a clinical extension of Dr. Warren's laboratory studies on the pathologic physiology of hepato-splenic schistosomiasis and its relation to ammonia tolerance and hepatic coma. It is possible that PL 480 funds will become available for additional work in Brazil and Poland.

The Laboratory is often called upon for help and advice concerning prevention and control of parasitic infections which are major health problems in many parts of the world. Because of this, we attempt to maintain competence to deal not only with basic problems of parasitism but also problems of prevention and control of parasitic diseases. Two of our staff, Dr. Olivier and Dr. Berry, have served on the World Health Organization Expert Panel on Parasitic Diseases. Dr. Olivier served as WHO consultant, 15 January to 12 March, 1961, when he visited India to study a schistosomiasis focus near Bombay. Dr. Berry served as rapporteur of a WHO working group which met in Washington, 6-9 November, to develop guides for the identification of American planorbid snails involved in the transmission of schistosomiasis.

Excellent rapport exists between this Laboratory and the Laboratory of Germfree Animal Research. Collaborative projects on a number of problems in relation to parasitism, nutrition, serum proteins etc. in germfree animals have been very profitable. We also have good working relations with other Laboratories, such as LID, in the study of eosinophilic meningitis. The work with the Ophthalmology Branch, NINDB, on the [illegible] of *[illegible]* has been very rewarding. Similarly, the work on the [illegible]

obtained for as long as 35 days (122-A). Microfilariae were discharged from the females during the first 2 to 7 weeks, only when serum was present in the medium. Under these conditions for maintenance of the filariids, glucose was transformed quantitatively to lactate (123-A).

In the study of the development of *Nematospiroides dubius* in germfree animals, the nematode has now been carried through its entire life cycle three times in the absence of bacteria (122-B). Larvae from the feces of germfree mice have been cultivated axenically to the infective stage and then used to reinfect new germfree mice. It is noteworthy that infections thus produced in germfree animals are not as extensive as in conventional animals; worm burden, worm size, and egg-production appear reduced. This may represent an important lead in determining the factors responsible for alterations in host-parasite balance. The possibility of a steroid action, which is in some way dependent upon the microbial activity in the intestinal tract, is suggested by the differences between conventional and germfree animals in respect to the worm recovery in male versus female mice.

The work with *Angiostrongylus cantonensis* (122-C) that has been carried on in this Laboratory, following the discovery of this helminth in cases resembling eosinophilic meningoencephalitis in Hawaii, is basic to experimental production of the syndrome in laboratory animals. The work already demonstrates the susceptibility of indigenous slugs to *A. cantonensis* and allows for detailed morphological and physiological study. Adults of *A. cantonensis* have been kept alive *in vitro* for over one month (122-A).

The study on the relation of nutrition to schistosomiasis in Puerto Rico is almost complete (122-E). Liver function tests returned to normal in all schistosome-infected patients who were placed on the high protein, high caloric diet. Stibophen (Fuadin) levels were higher and maintained longer in the same patients.

Studies on the mechanisms which produce higher blood levels of stibophen in human beings on high protein high calorie diet have been accomplished in mice on complete semi-synthetic diets in which enhanced activity of stibophen is observed. The higher levels of drug are attributable to the acid-base relations of salts in the diet. With acidic salts Fuadin activity is enhanced. Similarly the efficacy of antimony potassium tartrate, antimony (III) sodium meso-2, 3-dimercaptosuccinate (Astiban) and pararosaniline pamoate for killing mature *Schistosoma mansoni* in mice has been found to be increased [illegible] 10 times in mice maintained on the semi-synthetic diet compared to mice on the commercial pellet diet (122-D).

Biochemical studies on *Trypanosoma cruzi* (123-A) have revealed the fixation of carbon dioxide by this organism, with the incorporation of the CO_2 into succinic acid. Degradation of succinic and acetic acids from incubates containing C^{14} labeled glucose has established that most of the carbons of these acids are derived from glucose. Preliminary fractionation of the phospholipids of *T. cruzi* has yielded serine and ethanolamine as well as an unidentified ninhydrin-positive fraction. CO_2-fixation is known to occur in a number of free-living and parasitic protozoa, and some parasitic helminths. It appears that CO_2 functions as a metabolite as well as appearing as an excretory product.

The studies on the loss of sugars from *Cysticercus fasciolaris* and *Taenia taeniaeformis* in sugar-free solutions and the absorption of glucose and galactose when these are available (123-A) are basic observations in work that is being continued on the kinetics of absorption of nutrients through the cestode surface. It can be expected that further work in this direction may lead to demonstration of active and selective transport. In connection with the cestode studies it is of interest to note that the data show the metabolism of larval and adult worms to be proportional to fractional powers of the body weight intermediate between these characteristic for animals with increase in metabolic rate directly related to weight and for animals with increase in metabolism proportial to surface area (123-A). This was an unexpected finding because in tapeworms surface and body weight increase in the same ratio, and hence an increase in metabolic rate paralleling weight increase had been anticipated. Also, the quantitative, rather than qualitative, nature of differences in metabolism of adult and larval worms is noteworthy, since in other forms of parasites, e.g. trypanosomes, there are qualitive differences between various forms.

Additional work on the calcareous corpuscles of *T. taeniaeformis* in comparison with artificial mixtures of appropriate salts indicates that the dolomite structure must be preformed in some way in the corpuscles although they are definitely amorphous until heated. This is of considerable biological and geological interest (123-A).

Biochemical studies on intact rat liver mitochondria (123-C) have shown that a reversal of DPN-flavin-linked oxidative phosphorylation is involved in the reduction of acetoacetate to succinate, with an energy transfer equivalent to one high-energy bond per molecule of acetoacetate reduced. This energy can be supplied by one or both of the two terminal respiratory chain phosphorylations without the intermediary of extramitochondrial ATP. Oxaloacetic acid inhibits oxidation of succinate by DPN-depleted mitochondria. The inhibition is not of the classical competitive type but can be overcome by added ATP. Mitochondria inhibited by oxaloacetate gradually regain ability to oxidize succinate after the oxaloacetate is removed. These findings support concepts previously advanced that there is a compartmentation of substrate-level phosphorylation within mitochondria. This work, on basic biochemical pathways, is of general importance and obviously may be useful in explaining mechanisms for succinate metabolism in parasites.

The potentiation of encephalomyocarditis virus by *Trichinella spiralis* in mice (121-A) is closely related to the size of the worm inoculum and the time in the worm infection that the virus challenge is given. A similar phenomenon, of enhanced multiplication of Coxsackie A-9 virus in murine muscle, is again related to *T. spiralis* infection. The studies on *Taenia taeniaeformis* infection in mice (121-A), which seem to equate natural resistance with an accelerated response of acquired resistance, are of general interest. The demonstration of a possible immune tolerance in animals receiving *T. taeniaeformis* antigen shortly after birth, is of considerable significance; this phenomenon has not been demonstrated previously in relation to parasites.

Continued work on the pathology of schistosomiasis (124-B) has revealed differences in response among mice, hamsters, and multimammate rats (*Mastomys*) as to the vascular lesions seen in the liver. Mice and *Mastomys* develop more lesions than hamsters. The explanation for this may be that the lesions are at least partly due to allergic reactions and the hamster is notably less reactive in this respect. The development of hepatosplenic schistosomiasis occurs in mice even with very small worm burdens; a longer period of time is required for the syndrome to appear (125-D). The cor pulmonale picture produced in schistosome-infected mice by partial portal vein ligation provides another experimental model for studying clinical schistosomiasis (125-D).

Toxoplasma gondii has been found in the ovaries and oviducts of healthy chickens (125-A). The organism is present in the cyst form that can survive digestion. The possibility that it can be found in shelled eggs is important in regard to the epidemiology of toxoplasmosis. Also, it has a technical importance relative to the use of chick embryos for cultivation of viruses and rickettsiae.

In sheep, resistance to congenital transmission of toxoplasmosis is manifest only against moderate challenges. No protection is manifested against high challenge doses. This is of interest in human medicine. Exposure to large numbers of *Toxoplasma* can occur in women of particular population groups that customarily enjoy raw meat, and under such circumstances it is possible that habitual abortion may occur. This has been reported from Germany but not from the United States (125-A).

The failure to find *Toxoplasma* in cattle in New Zealand, while sheep are heavily infected in the same environment, is of particular interest relative to the means of transmission among herbivores (125-A).

Electron microscopy of *Toxoplasma* has revealed a schizogonic reproductive process, sometimes with the production of two filial parasites within a parent cell and sometimes apparently with the production of small rosettes. A third process, in which two organisms are involved, may represent longitudinal fission or a sexual fusion. These new observations lend support to the concept that *Toxoplasma* belongs among the Sporozoa of the Protozoa, probably in a new sub-class along with organisms such as *Besnoitia* and *Sarcocystis* (125-A).

Under the electron microscope, the formation and character of the cyst wall of *Toxoplasma* has been studied. The wall arises from interaction of the parasite and the host cell. The nature and development of the cyst are important in understanding chronic infection. Although some activity of cysts is evident in chronic infection, long-term treatment with pyrimethamine begun as early as three weeks after infection can produce some diminution in residual brain infection but cannot eliminate it (125-A).

Progress has been made in the fractionation of *Toxoplasma* antigens (125-B). Separation of hemagglutinating antigen on a hydroxyl apatite column yields an initial fraction that induces hemagglutinating antibodies but not dye test antibodies when injected into rabbits. This fraction has spectrophotometric activity indicating a high proportion of nucleoprotein, presumably

from the cytoplasm of the parasite. Attempts to get a "clean" cell wall preparation to induce dye test antibody formation in the absence of HA antibody have thus far been only partially successful, in that such fractions have stimulated dye test antibodies more rapidly. If the complete separation can be attained, we can conclude that dye test antibodies are produced against the cell wall and that hemagglutinating and complement fixing antibodies are stimulated by the intracellular components of the parasite.

Serial No. NIAID-120
1. Parasitic Diseases
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Administration and research planning and coordination.

Principal Investigator: Leon Jacobs

Other Investigators: Louis J. Olivier

Cooperating Units: None

Man Years (calendar year 1961):

Total	3½
Professional:	½
Other:	3

Project Description:

This project furnishes supervisory and administrative services to all research projects in the Laboratory, as follows:

Over-all evaluation of research plans and initiation of field projects; integration of laboratory research activities with clinical studies of the Clinical Center; editing scientific and technical reports and manuscripts; preparation of reports, budget estimates, and exhibits; supervision over personnel, travel, correspondence, and maintenance; requisitioning and supervision of equipment and supplies; maintenance of reference library, etc.; and consultatory services to individuals, academic and other organizations, and liaison activities with other branches of the Service and the Federal Government.

Research projects are reviewed and, in consultation with Section heads, changes in research program are considered and initiated. The emphasis is placed on fundamental aspects of medical parasitology; extensions of projects into the fields of clinical or practical preventive medicine are attempted only when the laboratory work has progressed to a point where a sound basis exists.

Serial No. NIAID-121-A
1. Parasitic Diseases
2. Ecology, Biology, and
Field Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Host parasite relations in worm infections in laboratory animals.

Principal Investigator: Dr. Louis J. Olivier

Other Investigators: Dr. Allen W. Cheever

Cooperating Units: None

Man Years (calendar year 1961)
Total: 2 1/3
Professional: 1
Other: 1 1/3

Project Description:

Objectives:

To study the characteristics of worm infections in laboratory animals; to learn how host defense mechanisms act to resist worm infections; to learn how resistance to worm infection may be enhanced or reduced; to study variation in resistance of host strains; to study "natural resistance" to worm infection; to study how a virus infection is affected by a simultaneous worm infection.

Methods Employed:

1. Taenia taeniaeformis infection: Produce standardized liver infections in mice by oral administration of eggs. Judge the results of experimental procedures by their effect on the size of the liver lesions and other parameters. Procedures include attempts to immunize actively with small worm inocula and passively with serum antibody, and also trials at the production of immune tolerance. Other variables studied are hormone imbalance due to cortisone, variation of diet, splenectomy, and administration of zymosan.

2. Simultaneous Trichinella spiralis and virus infections: Give standardized worm infections followed by challenge with virus. Measure the effect of the concurrent infections by various parameters including recovery of virus, pathology and symptomatology.

Major Findings:

1. Taenia taeniaeformis infection: Active immunization (by infection) rapidly produced complete resistance to reinfection in mice. This parallels previous observations in rats.

Passive administration of antibody resulted in a decrease in lesion size.

Cortisone administration and splenectomy produced an increase in lesion size. Mice thus treated produced much less antibody than did infected control mice. Attempts to correlate decreased resistance with anti-inflammatory effects of cortisone were unsuccessful.

A moderate degree of increased susceptibility (presumably immune tolerance) has been achieved in mice receiving antigen shortly after birth.

2. Simultaneous Trichinella spiralis and virus infections: Observation of the potentiation of encephalomyocarditis virus infection in white rats by Trichinella spiralis was repeated. It was demonstrated that the potentiation of virus pathogenicity is closely related to the size of the worm inoculation and the time in the worm cycle at which the virus challenge is given. Essentially maximal potentiation is obtained when the inoculum is about 20 larvae per gram of body weight and the virus challenge is given ten days after the Trichinella larvae are fed. This inoculum produces a heavy, but sub-lethal, infection. On the tenth day after inoculation the worms are migrating to the muscles in large numbers and there is pronounced myositis. If the inoculum is reduced to ten larvae per gram the potentiation is reduced and with an inoculum of five larvae per gram there is no potentiation of the virus activity. Virus potentiation is progressively reduced as the time of virus challenge is moved forward. When the virus is given ten days before worm inoculation the pathogenicity of the virus is not affected. Similarly, if the virus is given more than ten days after the worm infection the effect is diminished and if the challenge comes more than 25 days after worm infection there is little or no potentiation.

Cortisone given in an amount sufficient to kill some of the rats potentiated EMC virus pathogenicity to a much smaller extent than the optimal Trichinella infection. This is taken to indicate that potentiation of virus activity by Trichinella is not simply due to a generalized stress syndrome.

When T. spiralis infection in weanling mice was followed after ten days by challenge with Coxsackie A-9 virus the amount of virus in the muscle remained high for many days while the amount of virus in the muscle of control animals fell rapidly to a very low level. This seems to indicate that the worm infection favors multiplication of this virus in muscle.

Significance to the Program of the Institute:

The mechanism of so-called natural resistance remains obscure in most diseases. The lack of multiplication in the host of most helminths simplifies studies of the dynamics of host-parasite interaction. *T. taeniaeformis* offers a particularly attractive model for such studies, since all lesions are located in the liver and hence are easily evaluated morphologically and biologically.

The present data indicate that resistance in mice to *T. taeniaeformis* may be primarily related to rapidity and extent of antibody response and that in this case "natural resistance" may be simply an accelerated acquired resistance. The results with mice made tolerant to the worm antigen support this thesis.

Since immune tolerance to parasites has not been previously demonstrated, its production in the *T. taeniaeformis* - mouse system is of considerable general interest in addition to its implication for the present experiments.

Demonstration of a substantial effect of concurrent *Trichinella spiralis* infection on virus infection is very provocative and offers new opportunities to study both worm and virus pathogenicity. It is possible that other worm infections may affect virus invasiveness, virus replication, and virus pathology.

Proposed Course of the Project:

Attempt to demonstrate specific nature of protective serum substance involved in passive transfer of immunity in mice to *T. taeniaeformis*. Determine the relative production of antibody to *T. taeniaeformis* by various inbred mouse strains. Further study of immune tolerance to *T. taeniaeformis* in mice. Obtain additional evidence of the immune tolerance phonomenon. Explain the *T. spiralis* EMC relation further especially to learn where the virus resides in the muscle, to learn how the worm affects virus multiplication and action, and to learn whether other worms and certain tissue irritants can act similarly. To investigate other worm-virus infections.

Part B included Yes

Serial No. NIAID-121-C
1. Parasitic Diseases
2. Ecology, Biology, and
   Field Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: The biology and control of the snail intermediate hosts of the schistosomes.

Principal Investigators: Louis J. Olivier

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives:

To discover more effective means for controlling the snail intermediate hosts of the schistosomes. To study the biology of the snails with the aim of discovering means for their control. To study methods for the use of chemicals as molluscicides. To study chemicals having promise as molluscicides. To develop more effective means for study of snail poisons in the laboratory.

Methods Employed:

Snails, or their eggs, are exposed to selected chemicals and the destructive capability of each chemical is measured and recorded. Optimal conditions of exposure time, dilution, formulation, etc. are sought.

Field studies in endemic areas are conducted to learn the ecology and habits of the snails and to test molluscicides and other destructive measures against them.

Major Findings:

Refinements were made in the technique used for exposure of snail eggs to dissolved chemicals. Tests are now run with improved egg containers and at 27°C in a constant temperature box.

Serial No. NIAID-121-C
1. Parasitic Diseases
2. Ecology, Biology, and
   Field Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Olivier, L. and Barbosa, F. S.: Survival and weight loss of *Australorbis glabratus* kept at controlled relative humidities in the laboratory. Proc. Sixth Internat. Congr. Trop. Med. Malaria. 2: 22-37. (1961)

Olivier, L.: Field control of the schistosome vectors. Industry and Tropical Health, IV., Proc. Fourth Conference of the Industrial Council for Tropical Health, Harvard Sch. of Publ. Health. 102-111. (1961)

Honors: Louis J. Olivier served the World Health Organization, Division of Endemo-Epidemic Diseases as a consultant from 15 January to 12 March. He was sent to India to study the schistosome focus near Bombay.

Awards: None

Serial No. NIAID-121-D
1. Parasitic Diseases
2. Ecology, Biology, and
Field Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Investigation of intermediate hosts and vectors of human diseases caused by worms.

Principal Investigator: Elmer G. Berry

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1 1/2
Professional:	3/4
Other:	3/4

Project Description:

Objectives:

To investigate the distribution, life history, ecology, and susceptibility to infection of schistosome intermediate hosts. To develop more accurate methods for identification of the snails.

Methods Employed:

A large collection of snails collected from West, East and South Africa as well as from other schistosome endemic areas in the Near East and the Western Hemisphere has been preserved in alcohol and is being studied. Colonies of certain species are being maintained alive in the laboratory in order to determine their susceptibility to schistosome infection.

Major Findings:

From the large collection of planorbid and bulinid snails many dissections of organs, particularly the genitalia, kidney, and raduiae have been made. A number of species which were at one time thought to be distinct are now regarded as variations of the same species. Some, however, are believed to be new to science.

Significance to the Program of the Institute:

This knowledge is essential in understanding the range of the species of snail which serve as an intermediate host to schistosomes infecting humans.

Proposed Course of the Project:

It is anticipated that portions of this study will be ready for publication by June 1962.

Part B included Yes

Serial No. NIAID-121-D
1. Parasitic Diseases
2. Ecology, Biology, and
Field Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project: None

Awards: None

Honors: Dr. Elmer G. Berry was chosen as Rapporteur at the PASB/WHO Working Group which met in Washington, D.C., 6-9 November 1961 to discuss the "Development of Guidance for Identification of American Planorbidae Involved in Schistosomiasis".

Dr. Elmer G. Berry was reappointed as Honorary Curator of Mollusks in the Museum of Zoology, University Museums, of the University of Michigan for 1961-1962.

in cultures and having had no direct contact with a host have been furnished to The Department of Medical Zoology, Walter Reed, for fluorescent antibody study and for antigen preparation.

A technique was developed to obtain preparations of living microfilariae of *D. immitis* completely free of blood cells by treating blood with saponin followed by enzymatic digestion (Streptolysin O; trypsin; others) and saline washes. Such preparations have provided axenic inocula for assessment of nutritional requirements, and will be used as a source of antigen.

Sausage stages of *D. immitis* have developed *in vitro* in autologous, homologous and heterologous (human) blood, but not in the blood of rat or rabbit. Equivalent differentiation has also occurred in NCTC 109 plus horse serum, but only with microfilariae which had been treated with enzyme; Streptolysin O showed greatest activity. Maximal sausage stage development was comparable to three to four days of intracellular growth in susceptible mosquitoes.

Adult filariids, tentatively identified as *Tetrapetalonema* sp. were recovered from the cotton-topped Pinche (marmoset) for the first time, and microfilariae from those hosts were studied in tissue culture.

2. *Angiostrongylus cantonensis*. Adults derived from laboratory-infected rats have survived *in vitro* one month (still alive). Eggs deposited in culture have embryonated and hatched in certain media, allowing this process which normally occurs in the lung to be studied. Culture fluids containing metabolic products have been furnished to Walter Reed for tests with spinal fluids and sera from Tahitian cases of eosinophilic meningitis suspected of having a helminth etiology.

3. *Nematode coelomocytes and vitamin B_{12}*. Red pigment concentration previously reported to develop specifically in the coelomocytes of the "parasitic" stages of *Nippostrongylus muris* grown *in vitro* in the presence of B_{12} has also occurred in cultures of the free-living rhabditiform stages exposed to B_{12}.

Significance to the Program of the Institute:

The growth *in vitro* of parasitic helminths will provide an important tool with which to study the physiology and metabolism of these organisms, and should facilitate the isolation and preparation of metabolic antigens involved in the development of functional immunity.

Proposed Course of the Project:

Study of the survival and development of filariids *in vitro* will continue, with particular emphasis on the growth of the stages seen in the insect vector. Other suitable mammalian hosts for experimental work on filariasis will be investigated.

Several B_{12} antagonists, among which is a new type of anthelmintic, will be studied relative to their effect on pigment accumulation in coelomocytes in the presence of B_{12}.

Part B included Yes

Serial No. NIAID-122-A

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Sawyer, Thomas K. and Cheever, Allen W.: Some internal parasites of the cotton-topped Pinche (Columbian Marmoset), *Oedipomidas oedipus*, with a note on the survival, *in vitro*, of microfilariae of one of the parasites. Proc. Helminth. Soc. Wash. (In press.)

Awards: None

Honors: None

Serial No. NIAID-122-B
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: The development of helminths in germfree animals.

Principal Investigator: Paul P. Weinstein

Other Investigators: Thomas K. Sawyer

Cooperating Units: Laboratory of Germfree Animal Research, NIAID-116

Man Years (calendar year 1961)

Total	5/8
Professional	3/8
Other	1/4

Project Description:

Objectives:

To establish helminth infections in germfree animals so that problems involving host-parasite relations, nutrition, immune response, and pathology can be studied in the absence of other complicating organisms.

Methods Employed:

Larvae are reared to the infective stage under axenic conditions, and eggs and larvae are isolated from natural sources and rendered axenic; these are used to infect germfree animals.

Major Findings:

1. Additional data were obtained to confirm previous findings that among conventional mice males were more susceptible than females to infection with *Nematospiroides dubius*, whereas no such difference in susceptibility to infection was found in germfree mice. Experiments are now in progress using monocontaminated (*Escherichia coli*) germfree mice to assess the role of intestinal microbial associates in the matter of host sex and susceptibility to infection.

2. It has been possible to take *N. dubius* through three passages of germfree mice, the eggs from a particular passage being reared axenically *in vitro* to the infective stage to obtain larvae to infect the subsequent group of germfree mice. These data suggest that continued

passage in germfree animals yields infections which are lighter and abort more rapidly, compared to infections arising from such passage in conventional mice.

3. It has proved feasible to derive immature and adult *N. dubius* from germfree mice for axenic *in vitro* culture studies.

Significance of the Program to the Institute:

The germfree system has proved to be of great value in studying the role which microbial contaminants of the digestive tract play in susceptibility to infection with helminths, particularly in investigating conditions which influence natural resistance, host specificity and nutritional relations of parasite and host.

Proposed Course of the Project:

The role of various intestinal microbial associates with reference to susceptibility to infection with *N. dubius* will be studied, with an attempt to determine their metabolic role in this phenomenon. Steroid and steroid hormone levels in nematode-infected conventional, germfree and monocontaminated mice will be studied to determine whether microbial metabolism of steroids in the intestine may be involved in the susceptibility phenomenon.

Part B included No

Serial No. NIAID-122-C
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: The role of helminths in the etiology of eosinophilic meningitis and meningoencephalitis.

Principal Investigator: Paul P. Weinstein

Other Investigators: None

Cooperating Units: Laboratory of Infectious Diseases, NIAID-67-B

Man Years (calendar year 1961)

Total:	1/8
Professional:	1/8
Other:	None

Project Description:

Objectives:

To investigate experimentally the role of helminths in producing the syndrome of eosinophilic meningitis and meningoencephalitis reported for humans, particularly in the Pacific area.

Methods Employed:

Appropriate stages of helminths suspected of causing the above syndrome are used to infect laboratory animals. The development of the parasite, and the host response are studied.

Major Findings:

1. Infections of a monkey, rats, and guinea pigs at NIH with cestode (Trypanorhyncha) larvae recovered from the oceanic bonita from Tahitian waters gave rise to no pathologic changes or abnormalities.

Similarly, studies with larvae identified as Anisakis sp. from the ature (scad) and shipped from Tahiti to NIH gave negative results despite the fact that in Tahiti such larvae freshly isolated from fish had been found by Drs. Rosen and Myers to migrate in guinea pigs through the intestinal wall to the body cavity.

2. Worms dissected from the fixed brain tissue of a Filipino who had died in a mental institution in Hawaii with a diagnosis of eosinophilic meningoencephalitis were identified as young adults of *Angiostrongylus cantonensis*. This is the second report of infection in man with this parasite. The normal host of this worm is the rat. First stage larvae from naturally infected rats (*Rattus rattus*) from Hawaii have successfully infected Maryland slugs *Limax maximus* and *Deroceras reticulatum*. Third stage larvae obtained from both species of slugs have given rise to normal infections in white laboratory rats. Infections arising from as few as 40 larvae have been lethal to a mature rat due to extensive pulmonary damage following the deposition of eggs and hatching of larvae in the lungs.

A rhesus monkey given approximately 2000 infective larvae showed no behavioral change for two months thereafter, and at necropsy no worms were found and no lesions were seen on gross examination of the heart, lungs, and central nervous system; however, histological examination has not yet been made.

Significance to the Program of the Institute:

Eosinophilic meningitis particularly as seen in Tahiti appears to be correlated epidemiologically with the consumption of fish. The experimental infection of laboratory animals with helminth larvae derived from fish will provide data on the validity of this hypothesis.

The relation of *Angiostrongylus cantonensis* to human disease may contribute to the clarification of the confusing picture of CNS disorders seen in the Pacific area.

Proposed Course of the Project:

Study of the host-parasite relation of *A. cantonensis*, and its culture *in vitro* and physiology will be continued.

Part B included Yes

Serial No. NIAID-122-C

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B.   Honors, Awards, and Publications

Publications other than abstracts from this project:

Rosen, Leon, Chappell, Raid, Laqueur, Gert L., Wallace, Gordon D., and Weinstein, Paul P.: Eosinophilic meningoencephalitis of man caused by a metastrongylid lung-worm of rats. Journal of the American Medical Association (in press).

Honors: None

Awards: None

Serial No. NIAID-122-D
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Effects of nutrition on chemotherapy of parasitic diseases.

Principal Investigator: William B. DeWitt

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961)

Total	1
Professional	1/4
Other	3/4

Project Description:

Objectives:

To determine the influence of dietary factors on the efficacy of parasite chemotherapy.

Methods Employed:

Animals maintained on defined diets either deficient or enriched in specific components are exposed to experimental parasitic infections and are treated either in the prepatent or patent periods. The influence of the diet on treatment is determined by (1) the reduction of the number of parasites, (2) the condition of the parasites, (3) growth and survival of the host, and (4) pathological conditions developing in the host. When efficacy of the drug is found to be modified by dietary factors specific biological and chemical methods are used to determine mode of action.

Major Findings:

As previously reported for stibophen, the efficacy of antimony potassium tartrate, antimony (III) sodium meso-2,3-dimercaptosuccinate (Astiban), and para-rosaniline pamoate for killing mature *Schistosoma mansoni* in mice has been found to be increased up to 16 times in mice maintained on a purified semi-synthetic diet, compared to mice maintained on a commercial pellet ration. Further investigation of the influence of the host's diet on activity of stibophen showed that inorganic residues

of the diet have profound effects. When acid-salt residue greatly exceeds the basic-salt residue the activity of stibophen is enhanced. The reverse is noted when the basic-salts are in excess. Toxicity of the drug to the host is not similarly increased when an excess of acid-salts is given.

Significance to the Program of the Institute:

Over 200 million people throughout vast areas of the world suffer from schistosomiasis. To date, no satisfactory means of treatment has been found. With antimony potassium tartrate, which is extensively used to treat schistosomiasis, it has been estimated that from a million courses given annually in Egypt more than 2,000 people are lost due to drug toxicity. In Brazil it has been observed that antimony potassium tartrate is found to be completely ineffective in treating the infected population in certain villages but relatively good results are obtained in other villages located within 50 miles. Based on our experimental results it seems likely that dietary factors could account for these differences. If means could be found to assure success in the treatment of schistosomiasis much would be gained for the benefit of mankind.

Proposed Course of Project:

The studies on the effects of specific dietary components on drug efficacy will be continued with special emphasis on tracer studies.

Part B included Yes

Serial No. NIAID-122-D
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Luttermoser, G. W. and DeWitt, William B.: Enhancement of stibophen (Fuadin) activity against *Schistosoma mansoni* in mice by feeding purified semi-synthetic diets. Am. J. Trop. Med. and Hyg. 10: 541. (1961)

Honors: None

Awards: None

Serial No. NIAID-122-E
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Effects of improving the nutrition of malnourished people infected with Schistosoma mansoni.

Principal Investigator: William B. DeWitt

Other Investigators: None

Cooperating Units: School of Medicine, San Juan, Puerto Rico
Rio Piedras Hospital, Rio Piedras, Puerto Rico

Man Years (calendar year 1961):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

To evaluate the effects of providing malnourished people infected with Schistosoma mansoni with an enriched diet containing an abundance of animal protein.

Methods Employed:

Malnourished people suffering from schistosomiasis are given high-protein, high-caloric, vitamin-enriched food over an extended period so that the effects of the improved diet on their well-being and on the parasitic infections may be determined. Such factors as the following are observed before and at various intervals after the patients are given the enriched diet: (1) Nutritional status as determined by physical examinations and biochemical tests; (2) egg-production and viability of eggs of the parasite; (3) antibody levels; (4) skin sensitivity to schistosome antigens; (5) intestinal uptake of essential nutrients; (6) liver function as determined by BSP and thymol turbidity measurements; and (7) biopsy examinations. After the effects of the improved diet on the health of the patients have been determined, the patients will be given a course of treatment so that the effects of the improved nutrition of the efficiency of the drug can be determined. Suitable control patients are likewise to be studied.

Major Findings:

The first phase of the project in Puerto Rico showed that undernourished people suffering from schistosomiasis respond favorably to an enriched diet. Biochemical studies made on patients before they received the good diet revealed a high incidence of liver function impairment which returned to normal under the influence of the good diet.

The second part of the project was concerned with determining the effects of dietary improvement on the efficacy of stibophen therapy. It was found that following test injections of stibophen higher serum levels were produced initially and were maintained longer in patients on the high-protein diet than in patients receiving a protein-restricted diet. This remarkable effect may have been due to the balance of inorganic ions in the diet (see NIAID-122-D) as was shown in experimentally infected mice, or perhaps to dietary alteration in serum protein components that form complexes with antimonial compounds. On the basis of the blood level studies showing that effective concentrations of antimony are maintained for approximately 8 hours following i.m. injections of stibophen in patients on the enriched diet and for only about 4 hours in those on the rice and bean diet, it was decided to devise a new treatment schedule based on multiple daily injections instead of the two injections per week used in the standard course. The results of stool examinations, rectal biopsies, and rectal scrapings made during the 5-month post-treatment follow-up showed that with the exception of one patient on the enriched diet, complete cures were effected in all patients on both the good diet and the rice and bean diet. No severe side effects were noted. In a series of 10 patients on the low-protein diet given the standard course of stibophen, only one possible cure was obtained. It is believed that the high order of efficacy of the new treatment schedule overwhelmed any effect contributed by the enriched diet.

Significance to the Program of the Institute:

This project is a direct extension of our basic laboratory studies which indicate that the normal growth and development of schistosomes is prevented when the experimental host is given a deficient diet. Many of the worms remain sexually immature and are unable to produce eggs, which are the most important factors in the pathogenic mechanism of schistosomiasis. No information has been available prior to the present study concerning the interrelation of diet and schistosomiasis in humans. The remarkable increase in efficacy of stibophen under the new treatment schedule is of obvious importance since to date no satisfactory treatment of the disease has been found.

Proposed Course of Project:

It is of utmost importance that the information obtained from the Puerto Rican project be utilized in continued studies. Proposals for future work include: (1) Through the use of Public Law 480 funds in Brazil, conduct a study closely following the general plan of the Puerto Rican project. This study would have special significance since severe schistosomiasis occurs in Northwest Brazil where extreme malnutrition is also prevalent. (2) Conduct critical biochemical and pharmacological studies on the effect of nutritional factors on enhancement of anti-schistosome drugs. (3) Further explore the use of various regimens to maintain adequate therapeutic blood levels of anti-schistosome compounds.

Part B included Yes

Serial No. NIAID-122-E
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards and Publications

Publications other than abstracts from this project: None

Honors: Dr. DeWitt was invited to participate in the Gordon Research Conference on Food and Nutrition entitled "Nutrition and Infection". The conference was held at Colby Junior College in August 1961.

Serial No. NIAID-122-F
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Studies on the influence of dietary factors on experimental parasitic infections.

Principal Investigator: William B. DeWitt

Other Investigators: Paul P. Weinstein

Cooperating Units: Laboratory of Germfree Animal Research, NIAID-116

Man Years (calendar year 1961):

Total	1
Professional	1/4
Other	3/4

Project Description:

Objectives:

To determine the effects of nutritional factors on host-parasite relations with special emphasis on pathogenesis of parasitic infections.

Methods Employed:

Experimental animals maintained on defined diets enriched or deficient in specific components are exposed to parasitic infections and the course of the infection is followed. Such factors as the following are evaluated: (1) nutritional state of the host, (2) egg-production and viability of the parasite, (3) antibody levels, (4) blood-protein studies, and (5) pathological conditions developing in the host.

Major Findings:

(1) Mice infected with tape worms (*Hymenolepis nana*) or with pin worms (*Syphacia obvelata*) rapidly undergo self-cure when given a complete semi-synthetic ration containing 30 parts casein, 17 parts fat, 53 parts sucrose plus all essential vitamins.

(2) Similarly, mice fed the complete semi-synthetic diet and subsequently exposed to *Nematospiroides dubius* prove to be unsatisfactory in that the rate of infection is lower and marked suppression of development of the worms and egg-production were noted.

(3) Baseline serum protein values of germfree guinea pigs and mice were obtained in preparation for contemplated serologic studies on the pathogenesis of parasitic infections in germfree hosts.

Significance to the Program of the Institute:

Since parasitic infections occur most frequently in backwood areas of the world where nutritional problems are also prevalent, it is necessary to determine the relation between the nutritional status of the host and the role of parasites in the production of disease.

Proposed Course of the Project:

The present studies are to be extended to determine the specific nutritional factors in the semi-synthetic diet involved in producing adverse effects on the intestinal parasites of mice.

Part B included Yes

Serial No. NIAID-122-F
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications:

Publications other than abstracts from this project:

Newton, Walter L. and DeWitt, William B.: Nutrition and serum protein levels in germfree guinea pigs. J. Nutrition 75: 145. (1961)

Awards: None

Honors: None

Serial No. NIAID-122-G
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Studies on Entamoeba histolytica and other parasitic protozoa.

Principal Investigator: Louis S. Diamond

Other Investigators: Harry D. Baernstein
Lucy V. Reardon

Cooperating Units: Clinical Investigations Branch, NIDR
Biophysics Laboratory, Naval Institute for Medical Research

Man Years (calendar year 1961):

Total	3 1/2
Professional	1 3/4
Other	1 3/4

Project Description:

Objectives:

To study the physiology of Entamoeba histolytica, the mechanisms of its pathogenicity, immunity, factors in host susceptibility and differences in strain virulence. To study the nutritional requirements of Trichomonas tenax and other trichomonads in vitro. To study virulence of T. tenax in mice. To develop techniques for the preservation of parasitic protozoa by freezing and storage at low temperatures.

Methods Employed:

Microisolation procedures, monoxenic and axenic cultural techniques, population determinations with Coulter electronic cell counter. Immunological procedures including production of antigens from amoebae cultivated axenically and in association with Trypanosoma cruzi and animal inoculation studies. For preservation of protozoa at low temperatures, slow, fast and ultrafast techniques of freezing are used with preservation in dry-ice and liquid nitrogen.

Major Findings:

The reproducibility of the technique employed in the establishment of the first axenic culture of E. histolytica has been verified through cultivation of a second strain of the parasite. Moreover, the second strain, unlike the first, was a "wild strain" only recently isolated from a human case of amoebic dysentery.

The original strain of amoebae axenized slightly more than a year ago has been adapted to growing in a simplified version of the medium used for isolation. The simplified version is devoid of the chick embryo extract, vitamin preparation and the agar in the overlay.

In vitro studies on the nutritional requirements of three strains of axenically cultivated T. tenax revealed marked differences in growth rate in a standardized medium and ability of the flagellates to perpetuate themselves in the presence of a variety of chick embryo supplements.

Axenic Trichomonas hominis has been grown for the first time in a medium free of agar.

A strain of axenically cultivated T. tenax when inoculated intraperitoneally into normal mice failed to produce infection. Negative results were also obtained when the same strain was inoculated into mice which had been previously stressed by dosing with a series of intramuscular injections of hydrocortisone.

Antigens of E. histolytica have been prepared for the first time from amoebae grown under axenic conditions.

In a comparative study of the effectiveness of glycerol vs dimethylsulfoxide as a preservative of protozoa stored at -79 C, Trichomonas vaginalis was suspended in concentrations of 2.5, 5 and 10% dimethylsulfoxide and 5% glycerol. Survival was obtained in all preparations at 2 months, at 3 months in 5 and 10% dimethylsulfoxide only, and at 4 months in 10% dimethylsulfoxide only.

Using E. histolytica (associated with a Crithidia) and T. vaginalis as test organisms, the following results were obtained in a study comparing the efficiencies of liquid nitrogen and dry-ice as storage media for frozen protozoa.

	Survival in- Dry-ice (-79°C)	Liquid Nitrogen (-197°C)
E. histolytica	29 days	10 months (experiment still in progress)
T. vaginalis	4 months	6 months (experiment still in progress)

Serial No. NIAID-122-G
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Diamond, L. S.: Axenic cultivation of Entamoeba histolytica. Science 134: 336-337. (1961)

Awards: None

Honors: None

Serial No. NIAID-122-H
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Biochemistry of parasitic protozoa.

Principal Investigator: Harry D. Baernstein

Other Investigators: Louis S. Diamond
Lucy V. Reardon

Cooperating Units: None

Man Years (calendar year 1961)

Total	1/2
Professional	1/2
Other	0

Project Description:

Objectives:

To study the biochemistry of the parasitic protozoa; especially the intracellular enzymes and biochemical problems related to axenic and monoxenic cultivation.

Methods Employed:

Adaptation of electronic counting to protozoal populations.

Development of chemical procedures for the evaluation of protozoal populations.

The analysis of complex components of media, such as yeast extract, serum, etc.

Adaptation of starch block electrophoresis to the separation of enzymes of protozoa.

Major Findings:

The Coulter counter can be used to count Endamoeba invadens in monophasic medium. However, the organism is too big to allow sizing with either the 100 μ or the 150 μ aperture. It grows too slowly for daily counts.

Entamoeba histolytica in diphasic media with agar in the overlay cannot be counted electrically. Removal of agar is necessary. The agar also interferes with chemical methods for evaluation of populations by carrying over into the harvests significant amounts of medium components which interfere with chemical estimations.

The presence of malic dehydrogenase in T. vaginalis homogenates does not confirm the findings of other workers who have found no evidence of any citric cycle enzymes. Their failure to find them may have been due to the use of whole cells which are impermeable to these substrates.

Significance to the Program of the Institute:

A knowledge of the enzymes and metabolic pathways of the parasitic protozoa should help to differentiate various species, to understand their influence on the host, and to suggest chemotherapeutic measures.

Proposed Course of Project:

Analysis of complex media will be continued and better methods of evaluating populations developed. The isolation and purification of protozoal enzymes will be continued.

Part B included Yes

Serial No. NIAID-122-H
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Baernstein, H. D.: Malic dehydrogenase in Trichomonas vaginalis. J. Parasitol. 47: 279-284. (1961)

Honors: None

Awards: None

Serial No. NIAID-122-H
1. Parasitic Diseases
2. Host-Parasite Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Baernstein, H. D.: Malic dehydrogenase in Trichomonas vaginalis. J. Parasitol. 47: 279-284. (1961)

Honors: None

Awards: None

Major Findings:

Trypanosoma cruzi both under aerobic and anaerobic conditions fixes carbon dioxide which is incorporated into succinic acid. By means of degradation of succinic acid and acetic acid isolated from incubates containing radioactive glucose, it was established that most of the carbons of these acids are derived from glucose.

Preliminary fractionation of the phospholipids of Trypanosoma cruzi yielded as components serine and ethanolamine as well as an unidentified ninhydrin-positive fraction.

In sugar-free surroundings both Cysticercus fasciolaris and Taenia taeniaeformis excrete glucose and smaller amounts of two unidentified sugars. In sugar-containing solutions, rather large amounts of glucose or galactose are absorbed by the adult worms; this results in a sparing action on glycogen loss and sometimes, though so far not regularly, to an actual glycogen synthesis.

The differences in metabolism between larval and adult Taenia taeniaeformis are essentially quantitative in nature, the adult worms having a distinctly higher rate of metabolism than the larvae. The aerobic and the anaerobic metabolism of both larval and adult worms is proportional to fractional powers of the body weight intermediate between those characteristic for weight and surface proportionality.

Experiments additional to those reported last year confirmed that the carbonates of Taenia taeniaeformis calcareous corpuscles crystallize into dolomite when heated to 300° C. Artificial mixtures of appropriate salts did in most respects behave exactly like the corpuscles (formation of apatite, calcium oxide, magnesium oxide under suitable conditions), but never yielded dolomite, indicating apparently that the dolomite structure must be preformed in the corpuscles, although the latter are definitely amorphous when unheated.

Both males and females of Dirofilaria uniformis consume very large amounts of sugar and produce lactic acid in amounts about equivalent to the sugar consumed.

Significance to the Program of the Institute:

The studies on metabolism and chemical composition of parasites, intermediate hosts, and parasitized animals are essential for an understanding of the pathogenesis of parasitic diseases. They also contribute to the foundation which physiological and biochemical studies in general may eventually provide for a rational approach to chemotherapy.

Proposed Course of the Project:

The studies on succinate production have been terminated and the material is being prepared for publication. The studies on the composition of the phospholipids will be continued with the aim of identifying their various components.

An investigation on the anaerobic growth potentialities of culture trypanosomes will be initiated.

Studies on the metabolism of Taenia taeniaeformis will be continued with emphasis on the question of sugar absorption and glycogen synthesis. For the time being entire worms are used, but later homogenates will be employed.

Studies on the carbohydrate metabolism of Dirofilaria uniformis will be continued as material becomes available.

Part B included Yes

Serial No. NIAID-123-A

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

von Brand, Theodor: Neuere experimentelle Ergebnisse aus dem Gebiete der pathologischen Physiologie parasitarer Infektionen. Berichte der Physikalisch-Medizinischen Gesellschaft Wurzburg 60: 68-70 (1958-1959) Issued 1961.

von Brand, Theodor and Bowman, I.B.R.: Studies on the aerobic and anaerobic metabolism of larval and adult *Taenia taeniaeformis*. Exper. Parasitol. 11: 276-297 (1961).

von Brand, Theodor: Some research achievements and goals in protozoan biochemistry. Proc. I. Internat. Conf. Protozoology, Prague (In press).

von Brand, Theodor: Old and new observations on the chemical composition of *Trypanosoma cruzi*. Revista Inst. Med. Trop. Sao Paulo (In press).

von Brand, Theodor and Alling, David W.: Relations between size and metabolism in larval and adult *Taenia taeniaeformis*. Comp. Biochem. Physiol. (In press).

Awards: None

Honors:

Theodor von Brand was elected to honorary membership in the Mexican Society of Parasitologists.

Theodor von Brand was elected Vice President of the American Society of Parasitologists for 1962.

Theodor von Brand was elected as member of the Editorial Board of the Journal of Parasitology.

Theodor von Brand gave the introductory lecture on biochemistry of protozoa during the I. International Conference on Protozoology, Prague, Czechoslowakia (August 22-31, 1961).

Theodor von Brand lectured on April 7, 1961 before the Department of Microbiology, Yale University on the subject of disturbed liver metabolism during parasitic infections.

Serial No. NIAID-123-B
1. Parasitic Diseases
2. Physiology and Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Pathological physiology and histochemistry of parasitic diseases.

Principal Investigator: Dr. Teresa I. Mercado

Other Investigators: Dr. Theodor von Brand

Cooperating Units: Division of Biologic Standards, Laboratory of Viral Immunology

Man Years (calendar year 1961)

Total:	2¼
Professional:	1¼
Other:	1

Project Description:

Objectives:

To gain a better understanding by means of histochemical studies of the pathologic physiology of parasitic diseases. To elucidate factors which may modify the course of a parasitic infection and consequently influence the known pathologic physiology of the host induced by the infection.

Methods Employed:

Organ calcification induced by hytakerol (dihydrotachysterol) in normal rats and rats infected with Plasmodium berghei is studied histochemically by means of the von Kossa and the Alizarin Red S methods for the detection of calcium and the Alcian Blue technique for the detection of acid mucopolysaccharides.

In order to study a paralyzing factor occurring in the KBG 173 strain of Plasmodium berghei after passage through white mice, (1) conventional histological processing of brain and spinal cord tissues for study of pathology, (2) blood and tissue smearing, (3) staining with the Wright-Craighead and Giemsa stains, (4) animal inoculations, and (5) limited bacteriological procedures are used.

Major Findings:

Animals infected with Plasmodium berghei in general showed a lower incidence of calcification than the controls and this difference was more pronounced in those animals which received three and four doses than in those which received five and six doses of hytakerol. This quantitative difference was especially pronounced in respect to the coronaries and the great heart vessels. These findings differ markedly from those previously reported for Plasmodium praecox in canaries. An intercurrent Bartonella muris infection may have a modifying influence on metastatic calcification during Plasmodium berghei malaria.

The mouse-passed KBG 173 strain of P. berghei produces in white rats a marked paralysis (most commonly of the hind legs) 9 to 11 days following intraperitoneal inoculation of parasitized blood. In the terminal stages continuous twitching of the extremities with labored breathing, convulsions, and coma occur. Malaria is always present in the paralyzed animals but the parasite count is low (7 to 10 percent). Pathological study revealed punctate hemorrhagic areas in the white matter of the spinal cord (never in the gray) and of the brain in the more severe cases. Some demyelination of the nerve fibers also occurs. The degree of hemorrhage, i.e., involving the dorsal and ventral areas of the cord as well as the various levels is related to the severity of the paralytic condition. No specific agent has been uncovered so far.

Significance to the Program of the Institute:

Basic cellular studies in parasitic diseases are a necessity in trying to understand the interaction between parasite and host and may lead to the identification of specific parasite substances involved in pathogenesis. Histochemical studies provide means for localizing the sites of pathogenesis within tissues and furnish clues as to the physiological mechanisms that are altered.

Proposed Course of the Project:

The studies on metastatic calcification have been completed and the manuscript has been submitted for publication. Further studies on calcification are planned using a strain of avian malaria. An attempt will be made to define the properties of the paralyzing strain of Plasmodium berghei. This will depend on the ability to identify or to rule out a concomitant infection. Study of techniques involved in the histochemical demonstration of enzymes will be prosecuted as a preliminary to the study of enzyme distribution in the liver during various parasitic infections.

Part B included Yes

Serial No. NIAID-123-B

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

von Brand, Theodor and Mercado, Teresa I.: Histochemical glycogen studies on *Fasciola hepatica*. J. Parasitol., 47: 459-463, (1961)

Awards: None

Honors: None

Serial No. NIAID-123-C
1. Parasitic Diseases
2. Physiology and Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Biochemical mechanisms of energy metabolism in normal and parasitized animals.

Principal Investigator: Dr. Eugene C. Weinbach

Other Investigators: None

Cooperating Units: Department of Physiological Chemistry, Wenner-Gren Institut, Stockholm, Sweden

Man Years (calendar year, 1961)

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

The general objective is to conduct fundamental studies on the mechanism of energy metabolism in normal and parasitized animals. The specific objective has been to obtain information concerning the mechanism of phosphorylation occurring in the flavoprotein region of the respiratory chain.

Methods Employed:

Mammalian tissues are fractionated by mechanical disruption of the cells in suitable media and the subcellular elements (nuclei, mitochondria, microsomes, soluble fraction) are isolated by differential centrifugation. Mitochondria are fragmented further by various chemical and mechanical means to obtain structures bearing the electron transport system. These cellular and subcellular fractions are employed as biological catalysts to study electron transport, oxidative phosphorylation, and related exergonic reactions. Intramitochondrial substrates, nucleotides, and inhibitors of metabolism are determined by suitable enzymatic, chemical, radiochemical, and spectrophotometric techniques. Manometric and polarographic procedures are used to measure oxidation rates. Radioisotope and chromatographic techniques are used to investigate subtle metabolic changes not amenable to conventional chemical analyses.

Major Findings:

Intact rat liver mitochondria incubated under aerobic conditions in the absence of a phosphate acceptor catalyze a reduction of acetoacetate to β-hydroxybutyrate, coupled to the oxidation of succinate to fumarate. The reaction, which provides conclusive evidence for a substantial transfer of hydrogen from succinate to mitochondrial DPN, was found to be highly sensitive to amytal, dinitrophenol, antimycin A, cyanide, and in general, to conditions which suppress the formation or maintenance of high energy intermediates. Respiration with succinate as substrate in the absence of phosphate is stimulated by acetoacetate; the stimulation corresponds to ½ μatom of oxygen per μmole acetoacetate reduced. From these and related findings it is concluded that the reduction of acetoacetate by succinate in tightly coupled liver mitochondria involves a reversal of the DPN-flavin-linked oxidative phosphorylation; the energy required by this process is equivalent to one high-energy bond per molecule acetoacetate reduced. This energy can be supplied by one or both of the two terminal respiratory chain phosphorylations without the intermediary of extramitochondrial ATP, and the succinate-linked reduction of mitochondrial DPN does not involve the antimycin A-sensitive site of the respiratory chain.

By using DPN-depleted rat liver mitochondria to preclude formation of oxaloacetate from endogenous substrates, it was found that the preparations oxidize succinate at a high rate and that the addition of oxaloacetate (OAA) powerfully inhibits the oxidation. The OAA inhibition is not relieved by agents or treatments which remove the OAA (as would be expected if the inhibition were simply of the classical competitive type), but can be overcome by added ATP. Mitochondria impaired in their capacity to oxidize succinate by exposure to OAA (which is then removed) gradually recover their ability for succinate oxidation. Since the recovery phenomenon is sensitive to arsenate and arsenite and occurs in the absence of added substrate it is likely that it is due to a resynthesis of high energy intermediates from the oxidation of endogenous substrate still retained by these mitochondria.

These findings support the concepts previously advanced that there are two types of OAA inhibition of mitochondrial succinoxidase and that there is a compartmentation of substrate-level phosphorylation within the mitochondrion.

Significance to the Program of the Institute:

This investigation, because of its fundamental nature in elucidating one of the vital processes in all living cells, has obvious significance to biochemistry in general and provides a background for an understanding of the subtle biochemical changes in energy metabolism associated with parasitism. In addition, it provides a solid foundation for future studies of the complex bioenergetic mechanisms occurring in parasite forms.

Proposed Course of the Project:

An intensive effort will be made to complete the investigation of the mechanism of succinate oxidation and its activation by ATP in mammalian mitochondria. Specifically, it is intended to critically examine the fate of oxaloacetate, which inhibits this reaction, by the use of the hitherto unobtainable isotopically labelled compound. Further studies of the recovery phenomenon will be conducted in an attempt to assess the role of endogenous substrates in supplying high energy intermediates. Collaboration with the University of Uppsala will be implemented to study the effect of oxaloacetate on the nucleotide metabolism of mitochondria.

It is anticipated that this portion of the work involving mammalian mitochondria will be terminated during the next year and studies of the electron transport system and associated phosphorylations in parasites and/or parasitized animals will be initiated.

Part B included Yes

Serial No. NIAID-123-D
1. Parasitic Diseases
2. Physiology and Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Biology of trypanosomes.

Principal Investigator: Eleanor J. Tobie

Other Investigators: None

Cooperating Units: Applied Immunology Section, Laboratory of Immunology, NIAID-148

Man Years (calendar year 1961):
Total: 1 3/4
Professional: 3/4
Other: 1

Project Description:

Objectives:

To clarify the life cycle of *Trypanosoma rangeli* in relation to its vertebrate and invertebrate hosts: to determine factors which affect the ability of the invertebrate host to transmit *T. rangeli*.

Methods Employed:

Young nursling rats and *Rhodnius prolixus* are used as hosts. A colony of *R. prolixus* is maintained. One strain of *T. rangeli* is maintained in *R. prolixus* and several *in vitro*. *R. prolixus* are infected by feeding on infected rats which were infected by the bite of those capable of transmitting the infection or by inoculation of *T. rangeli* into the hemocoele. Observations are made on each insect as to sex, age when fed, stage in which infection develops, results of several infective feeds, duration of infection, etc. Culture material is grown and harvested for immunizing of rabbits. Fresh frozen sections are made of infected *R. prolixus* and stained with conventional stains, and the fluorescent antibody technic applied by both the indirect and direct methods.

Major Findings:

Almost twice as many male *Rhodnius prolixus* develop salivary gland infections and are capable of transmitting the infection than do females when infective feeds are begun in the first instar stage. Multiple

infective feeds appear to produce more infections and show that infection can develop in any stage. Infections persist throughout the life of the insect so that those which transmit it as nymphs also do so as adults.

Significance of the Program to the Institute:

These studies are providing information concerning the relation between a trypanosome and its invertebrate host which may well be applicable in cases of mixed trypanosome infections (e.g. *T. cruzi* and *T. rangeli*) because they shed light on the pitfalls possible in diagnosing such mixed infections. They are of epidemiological significance in relation to the finding of infected triatomids in the field. They also may well contribute to the elucidation of the question whether *T. rangeli* strains are really only strains of one and the same species or whether a number of closely related species occur in nature.

Proposed Course of the Project:

As a means of clarifying the life cycle of *T. rangeli* in the invertebrate host fluorescent antibody studies will be continued. Observations will also be continued on the factors affecting the ability of the invertebrate host *R. prolixus*, to transmit *T. rangeli* and one or two other triatome species will be tried as invertebrate hosts.

Part B included Yes

Serial No. NIAID-123-D

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Tobie, Eleanor J.: Experimental transmission and biological comparison of strains of *Trypanosoma rangeli*. Exp. Parasitol., 2(1): 1-9 (1961).

Awards: None

Honors: None

Serial No. NIAID-124-A
1. Parasitic Diseases
2. Ecology, Biology, and
   Field Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Effect of radiation on the snail hosts of *Schistosoma mansoni* and on schistosome cercariae.

Principal Investigator: Alina Szumlewicz (Visiting Scientist from Instituto Nacional de Endemias Rurais, Nucleo Central de Pesquisas, do DNERu, Rio de Janeiro, Brazil)

Other Investigators: Elmer G. Berry

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1 1/2
Professional:	1 1/4
Other:	1/4

Project Description:

Objectives:

To determine the radiosensitivity of all snail stages. To follow the biology of survivors of large doses of radiation. To determine the radiosensitivity of schistosome larvae as they develop in the snails.

Methods Employed:

Eggs of snails in all stages of embryonic development are exposed to radiation by an electron beam or by X-rays. The lethal dose for eggs is readily determined by inspection. Snails of various ages from hatching to maturity are also irradiated. For these both the lethal and sterilizing dose are determined. Survivors of irradiation are being observed for quantitative and qualitative reproductive capacity and for morphologic changes that can be attributed to irradiation.

Major Findings:

Fresh eggs can survive up to 4,000 roentgens. Somewhat older eggs will stand up to 5,000 or 10,000 roentgens. Just before hatching the eggs will tolerate up to 25,000 roentgens but their development is retarded.

Snails 2 to 11 days after hatching will withstand relatively high doses of radiation and resultant deaths are spread over a period of two weeks following irradiation.

The viability of free swimming cercariae of S. mansoni is affected by radiation doses of over 10,000 roentgens. From 90 to 100 percent of the cercariae exposed to that amount died in 24 hours.

Significance to the Program of the Institute:

The project relates to the interest of the Laboratory in the control of schistosomiasis. It is conceivable that radioactive wastes may be used with safety in endemic areas as an aid in the control of the snail intermediate hosts. The information being sought will assist in the determination of whether this approach deserves further attention.

Proposed Course of the Project:

It is anticipated that this project will terminate in June 1962.

Part B included No

Serial No. NIAID-124-B
1. Parasitic Diseases
2. Ecology, Biology, and Field Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Pathology of acute and chronic *Schistosoma mansoni* infection in the mouse, hamster, and multimammate rat (*Mastomys*).

Principal Investigator: Allen W. Cheever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	2/3
Professional:	1/3
Other:	1/3

Project Description:

Objectives:

The determination of the relation of hepatic vascular lesions of *Schistosoma mansoni* infection to host reactivity and to normal vascular structure. Determination of the interrelation of hepatic vascular lesions, host reactivity, portal vein pressure and hepatic blood flow in *Schistosoma mansoni* infections.

Methods Employed:

Mice, hamsters, and *Mastomys* are infected with *S. mansoni* and sacrificed at suitable intervals for determination of the following parameters: Portal pressure, worm burden and hepatic egg count, liver and spleen size, evidence of porto-systemic anastomoses, morphologic study of hepatic vasculature in serial sections of India ink-injected livers, and histology of the liver, spleen, kidney, intestine and lungs.

Major Findings:

Mice infected with *S. mansoni* show vascular lesions bearing some resemblance to those in human schistosomal liver disease. Unisexual infections produce no such changes.

Hamsters show much less reaction to S. mansoni ova than do mice and Mastomys. Hamsters also fail to develop the striking vascular liver lesions seen in mice and show only slight elevation of portal pressure, although many more eggs per gram of liver are present. No significant porto-systemic collaterals have been demonstrated in hamsters, though these are regularly present in mice. Data on infections in Mastomys are incomplete.

Significance to the Program of the Institute:

Our understanding of the pathogenesis of human schistosomiasis is fragmentary and explanations of liver pathology are speculative. Although numerous well documented histopathologic studies are available, none relates this pathology to the pathophysiologic picture only recently described in experimental animals. Experimental pathologic studies also show little relation to the disease in humans aside from the production of granulomata. The results presently available suggest that the larger granulomata present in the mouse and Mastomys may be responsible for differences in the vascular pathology and pathologic physiology observed. It is hoped that more chronic infections will reveal further differences of interest.

Proposed Course of the Project:

Continued study of chronic infections in the above species and of acute infections in Mastomys. Investigations under way in mice include the relation of the development of portal hypertension to hepatic blood flow and the development of collateral circulation, bromosulphthalein retention, morphologic changes, worm burden and egg count. If this study provides results of interest similar studies with Mastomys and hamsters are to be correlated with them.

Part B included Yes

Serial No. NIAID-124-B
1. Parasitic Diseases
2. Ecology, Biology, and Field Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Cheever, Allen W.: Hepatic vascular pathology in mice infected with *Schistosoma mansoni*. Archives of Pathology (in press).

Honors: None

Awards: None

Serial No. NIAID-124-C
1. Parasitic Diseases
2. Ecology, Biology, and Field Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Effect of gelatin on the rate of clearance of colloids from the blood.

Principal Investigator: Dr. Allen W. Cheever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961)

Total:	1/3
Professional:	1/3
Other:	0

Project Description:

Objectives:

To determine the mechanism by which some gelatin preparations affect the clearance of colloids from the blood.

Methods Employed:

Carbon clearance: Mice are injected with a colloidal suspension of carbon and the rate of disappearance determined by estimating photometrically the carbon concentration in serial blood samples.

Colloidal gold clearance: The technique is similar to that employed for carbon except that radioactive gold colloid is used and concentrations are determined by counting radioactivity.

Clearance of denatured human serum albumin: The albumin is denatured by heat, labelled with radioactive iodine (I^{131}) and clearance determined as for radio-gold.

Gelatin effect: Various gelatin preparations are either incorporated into the colloidal suspension or injected into the mice separately to determine their effect on carbon clearance.

Serial No. NIAID-125-A
1. Parasitic Diseases
2. Clinical Parasitology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Studies on toxoplasmosis.

Principal Investigator: Leon Jacobs

Other Investigators: Milford N. Lunde, Marjorie L. Melton, Anastasia M. Stanley

Cooperating Units: Ophthalmology Branch, NINDB

Man Years (calendar year 1961)

Total:	3½
Professional:	1 3/4
Other:	1 3/4

Project Description:

Objectives:

The accumulation of data on the occurrence of toxoplasmosis in man and animals, to gain an appreciation of its epidemiology; study of the biology of the proliferative parasite and the cyst to evaluate their importance in epidemiology and in chronic disease; investigation of factors in the host or parasite responsible for changes in virulence and for continued activity of Toxoplasma in the chronic infection; elucidation of the mechanism of cyst-formation; critical study of toxoplasmicidal agents against proliferative parasites and cysts; evaluation of factors responsible for pathogenesis of ocular toxoplasmosis.

Methods Employed:

Standard procedures of animal inoculation plus special techniques for injections into the eye. A microisolation apparatus is used for handling cysts. A digestion technique has been developed for processing relatively large amounts of tissue for isolating Toxoplasma. Tissue cultures are used for morphological and chemotherapeutic studies, and for attempts at growing cysts *in vitro*.

Major Findings:

Toxoplasma gondii has been found in a number of pools of oviducts and/or ovaries from healthy hens processed in a Baltimore plant. These

birds originated in poultry farms situated along the eastern seaboard. The organism is present in its cyst form; a digestion technique was used to concentrate the organisms in the tissues and proliferative parasites would have been destroyed by the treatment. Hence, Toxoplasma may be present in hens' eggs and a study of shelled eggs as well as tissues of hens is now under way to reveal this possible source of human infection.

In addition to residual infection in muscle and brain, sheep frequently have Toxoplasma cysts in the lung in the chronic infection.

A relative resistance to congenital transmission of toxoplasmosis was revealed in sheep with naturally or artifically induced immunity when the ewes were challenged during mid-pregnancy with Toxoplasma cysts orally or subcutaneously, or with moderate inocula of proliferative forms (1 x 10^5 or 10^6) administered intravenously or subcutaneously. The latter is in contrast to the lack of protection against high challenge doses (1 x 10^7) of proliferative forms.

A parasitological survey of 40 beef diaphragms collected at an abattoir in New Zealand failed to reveal any cases of Toxoplasma infection in cattle. This is of considerable interest, since sheep and cattle are frequently grazed on the same paddocks in New Zealand.

Electron microscopy has revealed that Toxoplasma gondii reproduces by at least two methods: schizogony, in which a number of filial organisms appear to be formed within a parent cell, with the production of tiny rosettes; and endodyogeny, in which two organisms are formed within a parent. The latter process can be considered a special form of schizogony involving the generation of only two merozoites. A third process, tentatively interpreted as longitudinal division, may be present; or the structures seen may represent a sexual fusion. These morphological observations lend support to the concept that Toxoplasma belongs among the Sporozoa of the Protozoa, probably in a new sub-class along with organisms such as Besnoitia and Sarcocystis.

Under the electron microscope, the formation and character of the cyst wall of Toxoplasma has been studied. The wall apparently arises from the combined activities of the parasite and the host cell. The encysted organisms contribute to the cyst wall material by means of vesicles in their cell membranes. Inter-organismal strands of membrane material are also formed; these connect with the wall. Inter-organismal protoplasmic connections also are seen.

The injection of antigen into mice with old chronic Toxoplasma infections elicits occasional parasitemias to a slightly greater extent than is found in controls.

Long-term treatment with pyrimethomine and/or sulfa drugs did not reduce the infectivity of the brains of mice infected one month earlier. Long-term treatment starting one or two weeks after infection produced radical cure. Similar treatment starting at three weeks after infection produced some diminution in residual brain infection.

Significance to the Program of the Institute:

Knowledge of the occurrence of toxoplasmosis in domestic and wild animals is necessary for understanding the epidemiology of the human infection. Already, our findings of Toxoplasma in the flesh of meat animals and in chickens indicate that the broad geographic serological surveys of human beings that have been performed in the past are not adequate; we need additional information from intensive studies of local populations, organized to provide data on socio-economic status, customs and habits of people, etc. Continued work is necessary to explain the mechanisms of transmission to herbivores and to vegetarian human beings such as Hindus, who do not even eat eggs.

Furthermore, it is necessary to relate all factors of the epidemiological picture to the kinds of human disease seen with Toxoplasma infection. For instance, there are reports from Germany and central Europe relating Toxoplasma to habitual abortion in human beings, a phenomenon which has not been seen here. If, because of the widespread German custom of eating raw meat, women can be repeatedly exposed, it is conceivable that breakdowns in their immunity, allowing repeated congenital transmission, may occur. Especially in the light of our New Zealand studies on sheep, we are aware of the possibility that massive challenges may result in congenital transmission in immune animals.

Basic study of the chronic infection in animals is important for obtaining an understanding of clinical observations on human disease. There is suspicion that chronic Toxoplasma infection may be important in systemic as well as in ocular disease, but we have little basis for this at present. Continued study of factors influencing cyst-rupture and parasitemia in chronically infected animals may furnish clues as to pathogenetic mechanisms in the chronic human infection. The effect of drugs oncysts in the chronic infection has corollary importance.

Additional information on the morphology of Toxoplasma and on the mechanism of cyst-production has basic importance in assigning Toxoplasma to a proper position in the systematic classification of parasitic protozoa. Knowledge of the cyst is also requisite for understanding chronic infection.

Proposed Course of the Project:

Attention will be paid to: The epidemiology of toxoplasmosis in domestic animals, both herbivorous and omnivorous; more detailed epidemiological studies of human population groups, if possible; the circumstances of cyst-formation and cyst-rupture in chronic infections; the relation of cysts and dormant parasites to chronic disease; the cultivation of cysts in tissue culture; the mechanism of proliferation of *Toxoplasma* in cells in tissue culture; the effects of various drugs on cysts and on activity of the parasite during chronicity; the effect of irradiation on *Toxoplasma* from the standpoint of sterilizing meat and producing forms which are incapable of cyst-production, the latter for immunizing purposes; the relation of immunity to the spread of challenge infections.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Jacobs, Leon: Experimental studies relating to ocular infection in toxoplasmosis. Documenta Ophthalmologica 14: 330-338 (1960)

Jacobs, Leon: Biological, clinical and epidemiological aspects of toxoplasmosis. Bull. Post-Grad. Comm. Med. U. Sydney (Australia) 17: 2-15 (1961)

Remington, J. S.; Jacobs, L. and Melton, M. L.: Congenital transmission of toxoplasmosis from mother animals with acute and chronic infections. J. Inf. Dis. 108: 163-173 (1961)

Remington, J. S.; Melton, M. L. and Jacobs, L.: Chronic Toxoplasma infection in the uterus. J. Lab. Clin. Med. 56: 879-883 (1960)

Jacobs, Leon: Toxoplasmosis. N. Z. Med. J. (in press).

Jacobs, Leon: Toxoplasmosis in man and animals. N. Z. Vet. J. (in press).

Jacobs, Leon: Ocular toxoplasmosis. J. Ophth. Soc. Austral. (in press).

Remington, J. S.; Melton, M. L. and Jacobs, L.: Induced and spontaneous recurrent parasitemia in chronic infections with avirulent strains of Toxoplasma gondii. J. Immunol. 87: 578-581 (1961)

Gavin, M. A.; Wanko, T. and Jacobs, L.: Electron microscope studies of reproducing and interkinetic Toxoplasma. J. Protozool. (in press).

Wanko, T.; Jacobs, L. and Gavin, M. A.: Electron microscope study of Toxoplasma cysts in mouse brain. J. Protozool. (in press).

Serial No. NIAID-125-B
1. Parasitic Diseases
2. Clinical Parasitology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Serology of toxoplasmosis.

Principal Investigator: Milford N. Lunde

Other Investigators: Leon Jacobs, Anastasia M. Stanley

Cooperating Units: None

Man Years (calendar year 1961)

Total:	2
Professional:	1 3/4
Other:	¼

Project Description:

Objectives:

The determination of the usefulness of immunological tests in diagnosis of toxoplasmosis in man and animals; the fractionation of Toxoplasma antigens and their identification with antibodies revealed by various tests.

Methods Employed:

The dye, complement fixation, and hemagglutination tests are used for serological studies. Sonication, chromatographic, biochemical, electrophoretic methods, and agar-gel techniques are used for fractionating and identifying antigens and antibodies. Rabbits inoculated with various fractions are used as sources of serum.

Comparative tests are run, with various procedures, on the sera of man and experimentally infected or naturally infected animals to obtain information on the relative usefulness of the various means of diagnosis.

Major Findings:

Sonication at 9kC for 15 minutes yields about five times as much hemagglutinating antigen as lysing in distilled water. The antigen resulting from sonication is also useful in the complement fixation test.

Fractionation of HA antigen on a hydroxyl apatite column yields an initial fraction that induces hemagglutination antibodies but not dye test antibodies when injected into rabbits. This fraction has spectrophotometric activity indicating a high proportion of nucleoprotein, presumably from the cytoplasm of Toxoplasma. If this is true, then we can conclude that antibodies are stimulated by HA antigen related to intracellular nucleoproteins while dye test antibodies are not, but possibly are from parasite wall antigens. However, attempts to get a "clean" cell wall preparation to reveal dye test antibody production in the absence of HA antibody have thus far been only partially successful. Ribonuclease-digested cell wall fractions have stimulated both dye and HA antibodies in rabbits, with a delay in the appearance of the latter. Fractionation of HA antigen by chemical means yields four components, all of which are antigenic but which are as unstable as crude antigen in the HA test. These fractions are separable in direct and indirect (inhibition) HA tests.

Significance to the Program of the Institute:

Analysis of Toxoplasma antigens improves our understanding of the course of the infection and the relative importance of different antibodies in immunity and in the hypersensitivity component of pathogenesis. It is of great importance in relation to the chronic ocular symptoms and possibly to chronic systemic manifestations of infection.

Toxoplasmosis is attracting more and more attention as an infection of clinical importance in both man and animals. The need for a practical serological procedure for diagnosis in routine laboratories may be met by the hemagglutination test developed in this Laboratory, but it is necessary to ascertain its value in all stages of infection in various hosts.

Proposed Course of the Project:

Continued work will be done on the fractionation of antigens, using sonication and possibly the Ribi cell fractionator if it becomes available as well as the chemical, electrophoretic, and chromatographic techniques. The relation of antibodies to particular antigenic components and to the course of infection will receive further study. Antigens derived from tissue culture preparations will be compared with those from other sources. An evaluation will be made of the direct agglutination technique, using parasites separated from cells and preserved in various ways. Similar preparations of Toxoplasma will be used for skin test antigens, in attempts to standardize them. Comparison of the various serological tests will be continued. The nature of non-specific heat-labile antibodies in animals will be investigated.

Part B included No

Serial No. NIAID-125-C
1. Parasitic Diseases
2. Clinical Parasitology

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Trichomoniasis

Principal Investigator: Lucy V. Reardon

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives:

Study of the pathogenesis of *Trichomonas vaginalis*; evaluation of the usefulness of purported trichomonicides.

Methods Employed:

Pathogenesis of trichomonads is tested in mice by intraperitoneal injection of concentrated organisms. Axenic cultures of *T. vaginalis* furnish the inocula.

Cultures of trichomonads in media containing graded concentrations of drugs, inoculated with standardized numbers of parasites, are used to assay trichomonicidal activity of the drugs.

Major Findings:

Two new strains of *Trichomonas vaginalis* were isolated and tested for virulence in mice. One of these proved to be highly pathogenic. The other, which represented a re-isolation from a patient from whom an earlier strain was obtained, varied considerably in pathogenicity from test to test; this result is consistent with previous experience with the other strain from the same patient.

A new purportedly trichomonicidal drug, metronidazole ("Flagyl") recently introduced in France, has been found to be completely effective against *T. vaginalis in vitro*, in concentrations as low as 0.01 mg/ml.

Serial No. NIAID-125-C
1. Parasitic Diseases
2. Clinical Parasitology

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Trichomoniasis

Principal Investigator: Lucy V. Reardon

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives:

Study of the pathogenesis of *Trichomonas vaginalis*; evaluation of the usefulness of purported trichomonicides.

Methods Employed:

Pathogenesis of trichomonads is tested in mice by intraperitoneal injection of concentrated organisms. Axenic cultures of *T. vaginalis* furnish the inocula.

Cultures of trichomonads in media containing graded concentrations of drugs, inoculated with standardized numbers of parasites, are used to assay trichomonicidal activity of the drugs.

Major Findings:

Two new strains of *Trichomonas vaginalis* were isolated and tested for virulence in mice. One of these proved to be highly pathogenic. The other, which represented a re-isolation from a patient from whom an earlier strain was obtained, varied considerably in pathogenicity from test to test this result is consistent with previous experience with the other strain from the same patient.

A new purportedly trichomonicidal drug, metronidazole ("Flagyl") recently introduced in France, has been found to be completely effective against *T. vaginalis in vitro*, in concentrations as low as 0.01 mg ml.

In lower concentrations, down to 0.001 mg/ml, it appeared to be at least inhibitory to the parasites, but some survival of organisms occurred. In comparison, a concentration of 0.07 mg/ml of Aureomycin was necessary for complete inhibition of the trichomonad.

Significance to the Program of the Institute:

Trichomoniasis is a clinical problem of considerable importance in the practice of many physicians. Studies on the pathogenicity of strains, chemotherapy, etc. are important to its control and management.

Proposed Course of Project:

The principal investigator has retired. Various parts of the project are being prosecuted by other workers in the Laboratory. (See 122-G).

Part B included Yes

Serial No. NIAID-125-C
1. Parasitic Diseases
2. Clinical Parasitology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Reardon, L. V., Ashburn, L. L., and Jacobs, L.: Differences in strains of *Trichomonas vaginalis* as revealed by intraperitoneal injections into mice. J. Parasitol. 47: 527-532. (1961)

Awards: None

Honors: None

Serial No. NIAID-125-D
1. Parasitic Diseases
2. Clinical Parasitology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: The pathophysiology of Schistosoma mansoni infection in the experimental animal.

Principal Investigator: Kenneth S Warren

Other Investigators: None

Cooperating Units: Walter Reed Army Institute of Research

Man Years (calendar year 1961):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

To reproduce in the experimental animal the various clinical syndromes seen in man infected with Schistosoma mansoni, and then to determine the pathogenesis and pathophysiology of the syndromes.

Methods Employed:

General techniques include: Infection of mice with varying numbers of cercariae of S. mansoni; preparation of unisexual cercariae and their use; worm perfusion and counts and organ egg counts. Surgical procedures are used to measure venous pressures in both the portal vein and the inferior vena cava and to establish portal collateral circulation in mice. Liver biopsies, portal pressures and spleno-portograms in monkeys infected with S. mansoni are also performed.

Major Findings:

From a study of infections in mice in which treatment was instituted to kill most of the worms, it was concluded that the eggs produced in bisexual infections are the major factor in the development of hepato-splenic schistosomiasis mansoni, that toxins or allergins produced by the worms may be a contributing factor, and that dead worms and malnutrition are probably only of ancillary importance.

It was also shown that mice harboring as few as one pair of egg-producing worms can develop the hepato-splenic disease although it takes four to five times longer.

Further studies revealed that treatment instituted after hepato-splenic schistosomiasis is fully developed results in the rapid reversion, towards the normal, of liver and spleen size and portal pressure. The lesions in the liver produced by the eggs decrease in size and many of them appear to be resorbed completely within 20-25 weeks following treatment.

Mice infected with both sexes of schistosomes were subjected to partial portal vein ligation. The portal-systemic collateral circulation that developed led to the passage of many of the eggs into the lungs rather than to the liver. These mice developed a syndrome somewhat similar to that of cor pulmonale occasionally seen in humans with schistosomiasis. The lung weight was greatly increased, there was cardiac dilatation and the peripheral venous pressure was increased by 300%.

Monkeys infected with S. mansoni develop acute alterations in liver function tests just at about the time that oviposition commences. They also develop hematological changes including an eosinophilia. Liver biopsies revealed eosinophilic granulomata in the liver, although no eggs were seen. The liver function tests reverted to normal after this acute phase of the disease ended.

Significance to the Program of the Institute:

The development of animal models for the various syndromes associated with schistosomiasis may enable us to determine the etiology and pathophysiology of these disease states. Our studies reveal that schistosomiasis alone, in the presence of good nutrition, can cause liver disease. Furthermore, schistosome eggs appear to be the major factor in the development of this syndrome. Recently strong claims have been advanced that dead worms are the cause of the liver disease of schistosomiasis and that treatment may be inimical. Our findings seem to refute this concept.

Proposed Course of the Project:

Clinical studies in Brazil on hepatic coma and hematology in cases of schistosomiasis, under the PL 480 program, are being arranged. This will be the main program during 1962. If possible, we plan to continue animal studies on the effect of schistosomiasis on liver blood flow, and cardiac studies in mice with cor pulmonale (electrocardiograms and vectorcardiograms); to attempt to set up animal models of cerebral schistosomiasis; and to study chronic schistosomiasis in certain monkey species.

Part B included Yes

Serial No. NIAID-125-D
1. Parasitic Diseases
2. Clinical Parasitology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Warren, K. S: The etiology of hepato-splenic schistosomiasis mansoni in mice. Am. J. Trop. Med. & Hyg. (In press)

Honors: None

Awards: None

Serial No. NIAID-125-E
1. Parasitic Diseases
2. Clinical Parasitology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Ammonia metabolism and toxicity in relation to liver disease.

Principal Investigator: Kenneth S Warren

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 1 1/2
Professional: 1
Other: 1/2

Project Description:

Objectives:

To study the biochemical pathway of ammonia toxicity and the possible relation between ammonia toxicity and hepatic coma. More specifically, to determine whether drugs which exacerbate hepatic coma or are used in its treatment effect ammonia toxicity; to test whether the susceptibility of animals to ammonia toxicity is altered by different types of liver disease.

Methods Employed:

Ammonia toxicity to mice is established by determining the intravenous LD_{50}. The modification of the LD_{50} by drugs, anti-metabolites and liver disease is then determined. Blood and brain ammonia concentrations, glutamine concentrations, and blood pH are also studied.

Major Findings:

Additional data were obtained on the effect of temperature alterations on ammonia toxicity. Hypothermia was protective; hyperthermia enhanced toxicity. The action of various compounds known to exacerbate hepatic coma or currently in clinical use in its treatment received further study relative to their effects on ammonia transport and toxicity and on ammonia and glutamine concentrations in the brains of mice. These

studies, together with the failure to enhance ammonia toxicity by the induction of liver damage by various means, suggest that ammonia is not the sole factor in hepatic coma, but that some biochemical defects in brain metabolism may be involved as well.

Significance to the Program of the Institute:

Since the liver is one of the principal sites of pathology in schistosomiasis, and since it is necessary to evaluate the importance of this helminthiasis relative to hepatic coma in endemic areas, the basic studies on ammonia have considerable significance in regard to the human disease.

Proposed Course of the Project:

Clinical studies in Brazil on hepatic coma in cases of schistosomiasis, under the PL 480 program, are to be performed with special reference to ammonia metabolism. These studies will be extensions of the experimental work reported here.

Part B included Yes

Serial No. NIAID-125-E
1. Parasitic Diseases
2. Clinical Parasitology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Warren, K. S and Schenker, S.: Brain ammonia during convulsions in thiamine-deficient mice. Nature. (In press)

Honors: None

Awards: None

LABORATORY OF PARASITE CHEMOTHERAPY

Summary 1

130 - Administration, Research Planning and Coordination 5
131 - Experimental Chemotherapy of Schistosoma Mansoni 10
131-A - Chemotherapy of Plasmodium Gallinaceum and P. Berghei Infections 12
131-B - Effect of Nutritional Status of the Host on the Chemotherapy of Malaria 14
131-C - Protein Synthesis of Plasmodium Berghei in Vitro and the in Vitro and in Vivo Binding of Chloroquine 17
132 - Studies in Human Malaria 20
132-A - Chemotherapy of Intestinal Parasites 24
132-B - Physiology of Parasites, with Special Emphasis on the Effect of Chemotherapeutic Agents 28
132-C - Virus-Parasite Associations 31
132-D - Development of Human Pathogens in Immature Insects 35
132-E - Insect Tissue Culture 38
133 - Studies of the Biology of Simian Malaria and of Human Malaria in Simians 40
133-A - Immunological Studies of Malaria Parasites ... 43
133-B - Tissue and Cell Cultures 46
133-C - Biochemical Studies of Simian Malarias 48
134 - Studies on Simian Malarias in Human Volunteers 50
134-A - Studies in Human Malaria 54
135 - Studies of the Vectors of Simian and Human Malarias 58
135-A - Study of Cross Transmission of Simian Malaria to Man Under Field Conditions 62
135-B - Systematic Studies of the Malaria Parasites of Monkeys and Apes of Malaya and Nearby Territories 64

Laboratory of Parasite Chemotherapy, NIAID

Summary Statement of Research Activities, Calendar Year 1961

The United States is committed as an active participant to world-wide malaria eradication. Our principal contributions to the effort arise from basic and clinical studies on the disease. In addition, we are committed to the same kind of investigations involving parasitic infections in general, with special emphasis on schistosomiasis and intestinal parasites. Because of the zoonotic character of simian malaria, as demonstrated here in 1960, and a large interest by the senior staff in overall problems of malaria, the main research effort during the year has been in that field. To that end, certain changes in personnel assignments have been made, space for one Section was remodeled, another relocated, and a new unit established.

Due to the mounting administrative load on the Chief and frequent requests for consultive advice from outside agencies, principally WHO, PAHO, and ICA (AID), Dr. Martin D. Young, formerly Head of the Section on Epidemiology, was named Assistant Chief of the Laboratory and also Head of the Section on Chemotherapy. He began his duties in Bethesda on April 3.

Dr. G. M. Jeffery, on assignment from the Columbia laboratory for study at Yale University, 1960-61 (M.P.H., June 1961), was named Head of the Section on Epidemiology and assumed charge of that installation in June.

Dr. C. G. Dobrovolny, while on a short-term assignment to Malaya, was named Head of the Section on Cytology in Chamblee, Georgia, and relieved Dr. Eyles on June 1.

Dr. Don E. Eyles was placed in charge of a new installation, the Far East Research Unit (FERU), in Kuala Lumpur, Malaya, and took charge there on June 21.

As part of the overall program for remodeling laboratory space in Bethesda, the Section on Chemotherapy was given better space in building 5, and an insectary provided.

The Memphis laboratory (Section on Cytology) was moved to Chamblee, Georgia, and work began there on April 1. Shortly after the move was completed, it became apparent that the insectary space was heavily impregnated with deldrin. The area could not be de-contaminated, and it became necessary, therefore, to erect an insectary building which became available on November 15.

Drs. Eyles and Dobrovolny had worked in Malaya on short-term assignments since August 1960. The venture had proved so productive it was decided to establish a quasi-permanent unit there under the Office of the Chief. Negotiations were entered into with the U.S. Medical Research Unit in Malaya, through the Director, Walter Reed Army Institute of Research, and the Malayan authorities with the result that FERU was established with permanent quarters at the Institute for Medical Research in Kuala Lumpur. The Unit is committed to a program embracing all aspects of the simian-human malaria problem.

MALARIA - HUMAN Plasmodium falciparum from Colombia, South America was found to be resistant to several members of the important 4-aminoquinoline group of drugs, i.e., chloroquine, amodiaquine, and hydroxychloroquine. Because of the wide-spread use of these drugs, such resistance, if prevalent, could be of major significance. Resistance to mepacrine was present also. Resistance has now been shown, by certain malaria parasites, to each of the important groups of synthetic antimalarial drugs developed in the last 30 years.

A drug (BW-377C54), with reported activity against human malaria similar to that of chloroquine, failed to cure P. falciparum. Also, the Colombia strain of P. falciparum appears to be resistant to this compound.

A study of the life-pattern of Venezuelan vivax malaria indicates that it has an intermediate-type latent period following treatment of the initial attack. This differs from the short-term (Chesson) or long-term (St. Elizabeth) latent period of other strains.

The chloroquine-resistant strain of P. falciparum contains a characteristic component which can be detected in hydrolyzed RNA. This suggests a chemical method for identifying resistant strains of malaria.

MALARIA - SIMIAN Man-mosquito-man transmission was accomplished consistently with two different strains (B & M) of Plasmodium cynomolgi, even with low gametocytemias. Man-mosquito-monkey transmission was accomplished with each strain also.

Infections produced by the inoculation of blood parasitized with the B or the M strain of P. cynomolgi exhibited no significant differences in clinical manifestations. However, when these infections were induced in volunteers by mosquito bites, several differences were noted; in the M strain, the duration and height of the fevers were greater, the tertian patterns were more numerous and typical, and splenomegaly more frequent.

FAR EAST RESEARCH UNIT
STUDIES IN MALAYA

In Malaya, approximately 33 per cent of pig-tailed macaques (Macaca nemestrina) and about 40 per cent of the long-tailed macaques (Macaca irus) are infected with a malaria. A new species of malaria was isolated from this latter species and was designated Plasmodium fieldi in honor of Dr. John Field. M. irus also harbored P. knowlesi and P. inui. Hepatocystis semnopetheci was discovered for the first time in M. nemestrina.

Malaria parasites were also demonstrated in Macaca irus and M. nemestrina leonina from Thailand and from the latter monkey from East Pakistan. Plasmodium knowlesi and P. inui, along with a species of Hepatocystis, were found in M. irus lacta from an isolated island off the east coast of Malaya.

A significant proportion of all three Malayan species of leaf monkeys (Presbytis cristatus, P. obscurus, and P. malalophos) were shown to be infected with malaria. Exact species determination of the parasites have not been made.

Anopheles hackeri was shown to be a natural vector of P. knowlesi and at least two, possibly three, tertian-type parasites so far unidentified. A. maculatus, A. sundiacus, A. leucosphyrus, A. umbrosus and A. letifer were found to bite simians but so far there is no evidence that any transmit malaria. Experimentally, it was shown that A. maculatus, A. sundiacus, A. philippinensis, A. kochi and A. hackeri are susceptible to infection with P. cynomolgi.

LIVER TISSUE CULTURE Organized liver tissue from monkeys has been maintained in tissue culture for four days with 50 per cent cell survival.

BIOCHEMICAL STUDIES Analysis of ribonucleic acid derived from Plasmodium gallinaceum, P. berghei, P. cynomolgi, P. inui and P. gonderi revealed the presence of nucleotides of the usual purine and pyrimidine bases: adenine, guanine, cytosine and uracil with adenine most abundant and cytosine the least abundant. In addition, nucleotides of hypoxanthine are found indicating the activity of adenosine deaminase.

Significant binding of chloroquine to a component or components in plasma, but not to pure serum albumin, was demonstrated. The degree of binding of this drug to blood cellular elements in vitro could be altered by a number of physical means. Antimalarial drugs, energy sources, and metabolic inhibitors were studied to determine their effect on the incorporation of amino acids into malaria protein. None of the antimalarial drugs had an effect on this process or on glycolysis.

Nucleic acid extracted from a pyrimethamine-resistant strain of P. gallinaceum hastened the development of resistance in a normal strain exposed to both drug and the extract. A decrease in chloroquine sensitivity of P. berghei has been observed following exposure of infected donor mice to 1,000 roentgens of X-radiation.

NUTRITION AND MALARIA Nutritional deficiencies that favor the development of peak parasitemia in mice infected with Plasmodium berghei include folic acid, pantothenic acid, and niacin.

Deficiencies suppressing parasitemia include para-aminobenzoic acid and riboflavin. Aminopterin, a folic acid antagonist, also suppressed peak parasitemia. Thiamine deficiency appeared to have no effect. Deficiencies were studied both in the absence and presence of suppressive levels of chloroquine. In only one case did deficiencies have a reverse effect; pyrodoxine deficiencies appeared to favor the parasite in the presence of the drug but suppressed the parasites in the absence of the drug.

MALARIA DRUGS In the course of testing 112 compounds against Plasmodium gallinaceum, two members of a new class of compounds (MJ 4862 and MJ 5399) were found active.

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MALARIA DRUGS In the course of testing 112 compounds against *Plasmodium gallinaceum*, two members of a new class of compounds (MJ 4862 and MJ 5399) were found active.

INTESTINAL PARASITES

Bephenium chloride, given for three or five days, was highly effective against hookworm infections and also removed 68 and 88 per cent, respectively, of *Trichuris trichiura* worms. A rural population heavily infected with *Ascaris* lost 90 per cent of their worms when given single doses of piperazine. Hookworm and *T. trichiura* infections were shown to persist for at least seven years in institutionalized mental patients.

Tapeworms (*Hymenolepis diminuta*) exposed to mepacrine (Atabrine) *in vivo* were resistant to the action of the drug *in vitro*. This is interesting to follow up because it suggests a type of resistance not based on selection but upon physiologic adaptation of individual organisms.

Evidence suggests that three anthelmintics, bephenium, mepacrine, and dichlorophen, are effective by inhibiting the enzymes located on the outer surface of tapeworms.

INSECT TISSUE CULTURES

Lepidopteran tissues have undergone growth or extended maintenance in culture. In cultures of caterpillar hemocytes, SLE virus has been maintained up to 10 days.

Prepupae and pupae fat body cells readily proliferate in hanging drop culture for 10-14 days; SLE virus persists in such cultures and may increase between the 6th and 8th day.

VIRUS-MOSQUITO LARVAE ASSOCIATIONS

Larvae of *Aedes aegypti* and *Culex pipiens* mosquitoes acquired SLE viruses from an experimentally contaminated aquatic environment. The virus persisted through the subsequent pupae stage and into the adult insect. There have been nine confirmed transmissions of the virus by bites of the *Aedes* and nineteen by the *Culex* mosquitoes.

VIRUS-PARASITE COMBINATIONS

Plasmodium gallinaceum reduced the quantity of circulating encephalomyocarditis virus in chicks. Additional experiments have continued to verify the role of *P. berghei* in the transport of SLE virus to the central nervous system in mice when the virus is given intraperitoneally.

SCHISTOSOMIASIS

None of 185 compounds tested against *Schistosoma haemotobium* was active. Schistosomiasis was reported for the first time in Lebanon.

Serial No. NIAID-130
1. Parasite Chemotherapy
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Administration, research planning and coordination

Principal Investigator: G. Robert Coatney

Other Investigators: Martin D. Young

Cooperating Units: None

Man Years (calendar year 1961):
Total: 3
Professional: 1
Other: 2

Project Description:

This project furnishes certain technical, supervisory and administrative services to all research projects in the Laboratory, as follows:

a) Over-all planning of research and the coordination of research activities in the various Sections of the Laboratory.

b) Integration of Laboratory research activities with clinical studies of the Clinical Center.

c) Supervision over personnel, maintenance, travel, and correspondence.

d) Requisitioning and supervision of equipment and supplies.

e) Preparation of reports, budget estimates, and exhibits and other public relations materials.

f) Maintenance of reference library, reprint files, card catalogues, and specimen collections.

g) Consultatory services to individuals, commercial organizations, nongovernmental organizations, Government agencies including liaison activities with other branches of the Service, foreign governments, and international agencies.

h) Reviewing, editing, and revising scientific and technical reports and manuscripts.

Progress on research projects is reported quarterly and, when necessary, are discontinued or revised in order to meet current objectives.

During the past year there were several changes in personnel assignments, space for one Section was remodeled, another Section re-located, a new Unit established, and impetus given to the overall research program of the laboratory.

1) Due to the mounting administrative load on the Chief and frequent requests for consultive advice from outside agencies, principally WHO, PAHO, and ICA (AID), Dr. Martin D. Young, formerly Head of the Section on Epidemiology, was named Assistant Chief of the Laboratory and also Head of the Section on Chemotherapy. He began his duties in Bethesda on April 3. Dr. G. M. Jeffery, on assignment from the Columbia laboratory for study at Yale University, 1960-61 (M.P.H., June 1961), was named Head of the Section on Epidemiology and assumed charge of that installation in June. Dr. C. G. Dobrovolny, while on a short-term assignment to Malaya, was named Head of the Section on Cytology in Chamblee, Georgia, and relieved Dr. Eyles on June 1. Dr. Don E. Eyles was placed in charge of a new installation, the Far East Research Unit (FERU), in Kuala Lumpur, Malaya, and took charge there on June 21.

2) As part of the overall program for remodeling laboratory space in Bethesda, the Section on Chemotherapy was given better space in building 5, and an insectary provided.

3) The Memphis laboratory (Section on Cytology) was moved to Chamblee, Georgia, and work began there on April 1. The move was made for two main reasons; a single insectary located at Chamblee would serve that installation and the Section on Clinical Studies, located at the Atlanta Prison, as well, and monkeys for simian malaria-human studies and for human malaria-simian studies could be housed close to the inmate volunteers at the prison. Shortly after the move was completed, it became apparent that the insectary space was heavily 'seeded' with deldrin. The area could not be de-contaminated, and it became necessary, therefore, to erect an insectary building which became available on November 15.

4) Drs. Eyles and Dobrovolny had worked in Malaya on short-term assignments since August 1960. The venture had proved so productive it was decided to establish a quasi-permanent unit there under the Office of the Chief. Negotiations were entered into with the U.S. Medical Research Unit in Malaya, through Colonel R. P. Mason, Director, Walter Reed Army Institute of Research, and the Malayan authorities with the result that FERU was established with permanent quarters at the Institute for Medical Research in Kuala Lumpur. The Unit is committed to a program embracing all aspects of the simian-human malaria problem.

5) Continued emphasis was placed on the fundamental aspects of other problems in tropical parasitology and chemotherapy. These included the zoonotic character of simian malaria, the culture of insect tissues, the tissue culture of mammalian malarias, the mechanism of drug action on parasites, protein synthesis by malaria parasites, the effect of nutrition on chemotherapy, drug resistance by parasites, chemotherapy of intestinal parasites, the carriage of viruses by parasites, acquisition of viruses by insect larvae and subsequent transmission by the ensuing adult.

Serial No. NIAID-130

The cooperative study with Dr. John Tobie, LI (Ser. No. NIAID-148), on the application of fluorescent antibody techniques to the staining of human and simian plasmodia has been continued. New immunological and biochemical studies involving different species and strains of malaria infections to man have been entered into with Dr. Sanford Kuvin, LCI (Ser. No. NIAID-20-C) and Dr. Tobie (loc. cit.).

Part B included - Yes

Serial No. NIAID-130

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Coatney, G.R. and Greenberg, J. Effect of a diet deficient in Factor 3, vitamin E and cystine on the course of *Plasmodium berghei* infections in mice. J. Parasitol. 47:601-604, 1961.

Coatney, G.R. Malaria. Cyclopedia of Medicine, Surgery, Specialties 8:801-825, 1961.

Gaudette, L.E. and Coatney, G.R. A possible mechanism of prolonged antimalarial activity. Am. J. Trop. Med. & Hyg. 10:321-326, 1961.

Coatney, G.R., *et al*. Report and Recommendations on Malaria: A Summary. Am. J. Trop. Med. & Hyg. 10:451-502, 1961.

Coatney, G.R., *et al*. Report of the Technical Meeting on Chemotherapy. Technical Reports Series, WHO. 1961.

Gaudette, L.E., Titus, E.D., and Coatney, G.R. Physiological disposition of pyrimethamine-H^3. Exper. Parasit. 11:152-155, 1961.

Krichevsky, M.I. Pyrimethamine inhibition of a pyruvate phosphoroclastic-type reaction in a marine pseudomonad. Arch. Biochem. Biophysics 93:25-29, 1961.

Schellenberg, K.A. and Coatney, G.R. The influence of antimalarial drugs on nucleic acid synthesis in *Plasmodium gallinaceum* and *Plasmodium berghei*. Biochem. Pharmacol. 6:143-152, 1961.

Honors and Awards relating to this project:

G. R. Coatney

Visiting Professor, Department of Preventive Medicine and Public Health, School of Medicine, Howard University.

Visiting Lecturer on Tropical Public Health, Harvard School of Public Health.

Visiting Lecturer on chemotherapy, Malaria Eradication Training Center, Kingston, Jamaica.

Related Director, Gorgas Memorial Institute of Tropical and Preventive Medicine, Incorporated.

Consultant to Chief, Section on Malaria Eradication, Pan American Sanitary Bureau.

Member of the Expert Advisory Panel on Malaria of the World Health Organization.

President, American Society of Tropical Medicine and Hygiene.

President, Tropical Medicine Association of Washington, D. C.

M. D. Young

Member, Editorial Board, American Journal of Tropical Medicine and Hygiene.

Consultant, World Health Organization for Rumanian assignment.

Serial No. NIAID-131
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Experimental chemotherapy of Schistosoma mansoni

Principal Investigator: William F. Cantrell

Other Investigators: None

Man Years (calendar year 1961):
Total: 1 1/3
Professional: 1/3
Other: 1

Project Description:

Objectives: To discover compounds active against Schistosoma mansoni.

Methods Employed: Mice are infected with cercariae of Schistosoma mansoni and treatment is begun after 35 days. Mice are examined 50 days after infection for the presence of dead worms. The liver and portal system are perfused and the live or dead worms are counted.

Major Findings: One hundred and eighty-five compounds were tested against Schistosoma mansoni. These compounds are designated by our numbers S 1698 - S 1882. The majority of the compounds were purchased, but a considerable number were supplied by Abbott Laboratories, Mead Johnson Co., Pharmacia (a firm in Uppsala, Sweden) and Cooper, Tinsley Laboratories of Harrison, New Jersey. The compounds represent a wide variety of chemical structures and many are analogous of physiological substances. They were all inactive.

Significance to Bio-medical Research and the Program of the Institute: Schistosomiasis is a serious disease occurring in large proportions of the populations of many countries of Africa and South America. Presently available drugs are toxic and not very effective.

Proposed Course of the Project: The project will be continued as part of the program of Dr. George W. Luttermoser upon his return to Bethesda in January, 1962.

Part B included - Yes

Serial No. NIAID-131
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Luttermoser, G. W., DeWitt, W. B. Enhancement of Stibophen (Fuadin$^{(R)}$) Activity Against Schistosoma mansoni in Mice by Feeding Purified Semi-Synthetic Diets. Am. J. Trop. Med. and Hyg. 10:541-546, 1961.

Azar, J. E., Luttermoser, G. W., and Schacher, J. F. First Report of a Focus of Schistosomiasis in Lebanon. Am. J. Trop. Med. and Hyg. 10:709-711, 1961.

Honors and Awards Relating to this Project:

For Dr. George W. Luttermoser:

Appointed Visiting Professor of Parasitology at the American University, Beirut, Lebanon for one year.

Serial No NIAID-131-A
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Chemotherapy of *Plasmodium gallinaceum* and *P. berghei* infections.

Principal Investigator: William F. Cantrell

Other Investigators: Richard L. Jacobs

Man Years (calendar year 1961):
Total: 2 1/3
Professional: 2/3
Other: 1 2/3

Project Description:

Objectives: To investigate drug activity and drug resistance in the malarias of laboratory animals.

Methods Employed: Standardized infections of *P. gallinaceum* in chicks and *P. berghei* in mice are treated with known antimalarials in combination with various physiologically active substances or with previously untested substances alone. In studies of drug resistance passage lines are treated repeatedly with minimum effective doses with or without X-ray treatment of the infected host animal at each passage.

Major Findings: In *P. berghei* a long series of passages (8 months) in mice treated with chloroquine, pyrimethamine or both chloroquine and pyrimethamine did not produce drug resistant strains. The procedure was modified so that relapses as well as primary infections were treated in the same mice whereupon a strain resistant to pyrimethamine emerged in both the series treated with the combination and the series treated with pyrimethamine alone, but no resistance to chloroquine resulted. The procedure was further modified by subjecting the donor at each passage to 1,000 roentgens of X-radiation to induce mutations. This has been done through four passages with a definite change in response to 30 micrograms per day of chloroquine, but no change in response to 60 micrograms per day.

In *P. gallinaceum* the influence of nucleic acid extracted from a pyrimethamine-resistant strain upon the development of resistance in a normal strain has been observed. The number of passages required to yield a resistant strain was reduced from ten to four by *in vitro* or *in vivo* exposure of normal parasites to nucleic acids prepared from resistant parasites.

The search for specific inhibitors of chloroquine activity has not revealed any clues about the mode of action of chloroquine. The substances tried were pyridoxine, riboflavin, pantothenic acid and flavin-adenihedinucleotide, all of which failed to inhibit the activity of chloroquine against P. gallinaceum.

One hundred and twelve compounds were tested for antimalarial activity and significant activity was found in two compounds obtained from Mead Johnson Co. on a confidential basis. These are designated by our numbers S 1834 and S 1836. They are derivatives of a heterocyclic ring system not found in any previously known antimalarial compounds. Among the negative compounds were many interesting analogues of naturally occurring purines, pyrimidines, amino acids and coenzymes.

Significance to Bio-medical Research and the Program of the Institute: The results obtained with drug resistant nucleic acid and with X-ray hold out some promise of the development of experimental models that may clarify the phenomenon of drug resistance which appears to be of increasing importance in the malaria eradication program.

The search for new antimalarial compounds appears to be the most direct approach to the problem of drug resistance.

Proposed Course of the Project: These studies will be actively followed during the next year.

Part B included - No

Serial No. NIAID-131-B
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Effect of nutritional status of the host on the chemotherapy of malaria.

Principal Investigator: Richard L. Jacobs

Other Investigators: None

Man Years (calendar year 1961):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: To determine the effect of altered nutritional status of the host animal on the course of parasitemia in experimental malaria infection, both with suppressive levels of antimalarial drugs and in the absence of drug therapy.

Methods Employed: Weanling NIH Swiss strain mice average from nine to ten grams each when placed on test diets. All animals are maintained in wire-bottom cages during a four to six week depletion period prior to infecting. A purified diet is given fresh daily and all groups are weighed three times each week during the depletion period for comparison of growth rates. In tests employing antimetabolites, the analog may be administered prior to, or at the time of infection. The effect of altered nutritional states are studied both in the absence and presence of suppressive levels of chloroquine. Infection is accomplished by intraveneous injection of one million parasitized red blood cells (P. berghei, NYU-2 strain). Smears are taken on day-4 and day-6 for evaluating the course of parasitemia.

Major Findings: Alterations in the nutritional status of mice have been shown to affect the course of parasitemia in experimental P. berghei infections. Of the various factors studied, para-aminobenzoic (PABA) produced the most striking effect on parasitemia. Although PABA is not required by the mouse for normal growth and reproduction, parasitemia is suppressed 95% in mice reared on a purified diet deficient in PABA. Very small doses of chloroquine resulted in complete suppression of parasitemia in PABA deficient mice.

The effect of other nutritional factors studied has been less striking, but interesting results have accumulated from repeated tests. Folic acid

deficiency favors the development of parasitemia in the presence or absence of chloroquine by approximately 40%. However, the administration of aminopterin, a folic acid antagonist, consistently suppresses parasitemia by about 70%.

Pyridoxine deficiency appears to suppress parasitemia in the absence of antimalarial drug therapy, but favors development of the parasite in the presence of suppressive doses of chloroquine. The course of parasitemia was not altered by the administration of desoxypyridoxine, a pyridoxine analog, in mice maintained on practical (pellet) diets.

Pantothenic acid deficiency has slightly, but consistently, favored the course of parasitemia either with or without suppressive levels of chloroquine. Riboflavin deficiency suppressed peak parasitemia in the presence or absence of chloroquine. Niacin deficiency resulted in a slight increase in peak parasitemia and thiamine deficiency produced little, if any, effect. The administration of 3-acetylpyridine, an analog of niacin, produced slight, and variable, responses in mice maintained on a practical diet.

Significance to Bio-medical Research and the Program of the Institute: The correlation of metabolic interrelationships between the host and parasite in the presence and absence of chemotherapy should yield basic information needed to elucidate the mechanism of action of antimalarial drugs and to better understand the physiology of the parasite.

Proposed Course of the Project: The project will be continued along the general line described above. Additional dietary factors will be investigated along with other antimetabolites.

Part B included - Yes

Serial No. NIAID-131-B

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Jacobs, R. L. and Cantrell, W. F. An evaluation of antimalarial combinations against Plasmodium berghei in the mouse. J. Parasitol., in press.

Serial No. NIAID-131-C
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Protein synthesis of *Plasmodium berghei in vitro* and the *in vitro* and *in vivo* binding of chloroquine.

Principal Investigator: Leonard Friedman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: To determine the relationship between protein synthesis and carbohydrate metabolism of *P. berghei* and to determine the effect of antimalarial drugs on this relationship.

To study some of the variables involved in the binding of chloroquine to proteins and cells.

Methods Employed: Binding of chloroquine to protein was studied by dialysis equilibrium. Chloroquine assayed by a fluorometric technique. The *in vivo* binding of chloroquine to blood cell elements was studied by analyzing the separated cells and plasma of rabbits, chicks, and monkeys administered 10 mg of chloroquine per kg. body weight.

Protein synthesis was studied by following the *in vitro* incorporation of C^{14} amino acids into parasite protein. Glucose, pyruvate, and lactate was assayed by established enzymatic and chemical procedures.

Major Findings: SKF 525-A administered to monkeys orally subsequent to the administration of chloroquine raises the plasma level of the latter drug above that of its initial concentration. This effect was demonstrated also *in vitro* using livers from chloroquine-drugged rabbits.

It was not possible to demonstrate binding of chloroquine to bovine or human serum albumin by dialysis equilibrium although by this same technique,

50% rabbit serum was shown to bind about 20% of the chloroquine present (1 mg/ml). SKF effected no change in the degree of binding.

Chloroquine could be released from rabbit blood cells by (1) storing the whole blood at 30°C (but not at 5°C) for 24 hours, (2) heating at 60°C for 10 minutes, (3) freezing and thawing the whole blood or (4) adding to the whole blood plasma of heated non-drug containing blood. There was no apparent difference between the chloroquine-binding capacity of malaria-parasitized and normal chick red cells in vitro. The blood and plasma of malaria-infected chicks contained a higher concentration of chloroquine than did normal chicks receiving the same dose of chloroquine per body weight.

The in vivo administration of chloroquine increased the resistance of malaria-parasitized and non-parasitized chick red cells to lysis by saponin in vitro.

Neither chloroquine, pyrimethamine, mepacrine, primaquine, nor quinine had any effect on the in vitro utilization of glucose or the incorporation of C^{14} amino acids into protein by P. berghei. 10^{-5}M puromycin inhibited the incorporation by about 80% while 8-azaguanine and chloramphenicol (both known to inhibit protein syntheses in bacteria) had no effect on this process.

The in vitro incorporation of C^{14} amino acids into parasite protein virtually ceased after one hour while the utilization of glucose and the production of lactate continued for about 2 hours more. The energy derived from terminal respiratory cycle was apparently an unimportant source for the incorporation since pyruvate could not be used as an energy source, and the incorporation was not affected by a nitrogen atmosphere or 2,4 dinitrophenol (an oxidative uncoupler). 4×10^{-2}M oxamic acid (an inhibitor of lactic acid dehydrogenase) inhibited the incorporation by about 35% while 0.03% 2-deoxyglucose (a glycolytic inhibitor) inhibited the incorporation by almost 100%. 1.2×10^{-1}M sodium pyruvate completely inhibited the incorporation. Of the following compounds, glucose, galactose, fructose, lactose, sodium pyruvate, maltose and glycerol only glucose, fructose and maltose were significantly utilized as energy sources for the incorporation.

Significance to Bio-medical Research and the Program of the Institute: It is important to gain knowledge of the binding properties of chloroquine since this may not only provide clues as to its mode of action but will also assist in devising methods of prolonging and/or potentiating the action of chloroquine in the body.

By studying the relationship of the synthesizing activities to the energy-producing systems of malaria, we may gain insight into specific procedures which may differentially interfere with this relationship while not affecting those of the host.

Finally the effect of antimalarials must be studied in view of the existing knowledge that some of these drugs bind nucleic acids and that nucleic acids are involved in protein synthesis.

Proposed Course of the Project: Protein synthesis of P. berghei will be studied in cell-free systems and this process compared with those in bacteria and mammalian cells. Attempts will be made to isolate enzymes and other components of this system.

The in vitro and in vivo incorporation of other metabolites into malaria fractions will be examined as well as the effect of antimalarial drugs on these systems.

The binding of chloroquine to cell components will be further explored with a view toward discerning if other materials besides nucleic acids and proteins are involved in the binding and if the binding can be affected in vivo by enzymes and other drugs.

Part B. included - No

Serial No. NIAID-132
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Studies in Human Malaria

Principal Investigator: Martin D. Young

Other Investigators: William E. Collins, Geoffrey M. Jeffery, and Jimmie C. Skinner

Cooperating Units: South Carolina State Hospital

Man Years (calendar year 1961):

Total:	2 3/4
Professional:	3/4
Other:	2

Project Description:

Objectives: To add to the biological knowledge of malaria by observations and experiments on induced malaria. To study the development, characteristics, inheritance of resistance to drugs by the parasites. To determine the sporontocidal activity of antimalarial drugs and how developed resistance influences transmission by mosquito vectors. To determine if the development of resistance to insecticides by mosquitoes alters their ability to transmit malaria. To serve as probably the only source in the nation for established strains of malaria for use on a nationwide basis for therapy of neurosyphilis, nephrosis, Parkinsonism, and for experimental malaria studies.

Methods Employed: Malaria is induced in neurosyphilitic patients and in volunteers under normal and experimental conditions. The patent infections are challenged by drugs. When resistance occurs measurements are made of its characteristics. Mosquitoes are allowed to bite patients with induced infections before and at intervals after drug administration. The resulting infections in the mosquitoes are correlated with the presence or absence of drug resistance of the malaria infection in the human host. Insecticide-resistant and normal mosquitoes are fed simultaneously for comparison of ability to transmit malaria.

Major Findings: A Colombia, South America strain of *Plasmodium falciparum* was found to be resistant to normal (1.5g) and above normal (2.1g) doses of chloroquine. Cross-resistance was demonstrated to other 4-aminoquinoline drugs, i.e., amodiaquine and hydroxychloroquine. The resistant quality was present after mosquito transmission.

Weekly doses of chloroquine and amodiaquine normally effective against other strains of malaria failed to suppress this strain of malaria. Cross-resistance to mepacrine (Atabrine) was suggested by relapses occurring shortly after treatment.

This strain of malaria appeared to respond normally to pyrimethamine, proguanil, and quinine, indicating no cross-resistance between them and the 4-aminoquinoline drugs.

Two strains of Anopheles quadrimaculatus mosquitoes susceptible to insecticides were compared with a strain resistant to dieldrin in regard to infectivity by P. falciparum (Colombia strain). No significant differences were found indicating a lack of relationship between dieldrin resistance in A. quadrimaculatus and susceptibility to infection by P. falciparum (Colombia strain).

Post drug studies on the infectivity of P. falciparum (Colombia strain) to mosquitoes were made using pyrimethamine (50 mg dose) and A. freeborni mosquitoes. In one instance, the parasites became infective to mosquitoes 14 days post drug. In all other cases, however, this treatment prevented development of oocysts in the mosquitoes.

Comparative studies were made on the infectivity of P. falciparum (Colombia strain) to A. quadrimaculatus and a strain of A. albimanus from San Salvador. The A. quadrimaculatus were more susceptible to infection both in regard to per cent infection and to gut infection index than the A. albimanus.

Studies have been made on the use of a new additive in the preservation of frozen malarial parasites. The results thus far have indicated that dimethyl sulfoxide is a suitable substitute for glycerine in the long-term preservation of P. berghei and P. gallinaceum.

Significance to Bio-medical Research and the Program of the Institute: Drugs are playing an increasingly important role in malaria eradication programs throughout the world. The 4-aminoquinoline drugs are the most important ones for suppressive and therapeutic use and are widely employed. The finding of resistance to the 4-aminoquinolines, as well as cross-resistance to mepacrine, indicates that resistance may occur in other malaria strains in other areas. This could be of major significance if resistance to these drugs becomes more widespread. Resistance or cross-resistance has been demonstrated by malaria parasites to all of the important groups of synthetic antimalarials developed in the last 30 years i.e., the 9-aminoacridines, the 8-aminoquinolines, the diaminopyrimidines, and the biguanides. Additional information is needed on the biology of the parasite especially in relationship to drugs.

The comparative ability of insecticide-susceptible and insecticide-resistant mosquitoes to transmit drug-susceptible and drug-resistant parasites needs to be determined, especially to indicate whether the change in the vector or parasite has significance in the epidemiology of malaria.

Proposed Course of the Project: To determine more fully the nature and extent of the resistance of malaria parasites to the synthetic antimalarial drugs. To investigate the cause of resistance, and probable ways to avoid or overcome it. To consider the exploration of new groups of compounds which may be effective therapeutic agents. Further investigate the ability of insecticide-susceptible and insecticide-resistant mosquitoes to transmit drug-susceptible and drug-resistant parasites. Continue to obtain basic information, especially epidemiological and host-parasite relationships on induced human malarias, both domestic and foreign. Important strains of malaria will be added to those now in the local repository which apparently is the only existing one for the preservation of malaria.

Part B included - Yes

Serial No. NIAID-132

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Jeffery, G. M. Research Note. Inoculation of Human Malaria into a Simian Host, *Macaca mulatta*. J. Parasit. 47:90. 1961.

Young, M. D., and Burgess, R. W. The Infectivity to Mosquitoes of *Plasmodium malariae*. Am. J. Hyg. 73:182-192. 1961.

Young, M. D., and Moore, D. V. Chloroquine Resistance in *Plasmodium falciparum*. Am. J. Trop. Med. and Hyg. 10:317-320. 1961.

Young, M. D. Amodiaquine and Hydroxychloroquine Resistance in *Plasmodium falciparum*. Am. J. Trop. Med. and Hyg. 10:689-693. 1961.

Young, M. D. The National Institutes of Health Laboratory at Columbia, South Carolina. Bull. Assn. Sou. Biol. Vol. 8, No. 4, October 1961.

Young, M. D. Chemotherapeutic Agents and Malaria Eradication. Proceedings of Sixth International Congresses on Trop. Med. and Mal. (In Press)

Garnham, P. C. C., Jeffery, G. M., and Young, M. D. Preservation of Strains of Malaria Parasites. Proceedings of Sixth International Congresses on Trop. Med. and Mal. (In Press)

Young, M. D. Failure of Chloroquine and Amodiaquine to Suppress *Plasmodium falciparum*. Transactions of the Royal Soc. of Trop. Med. & Hyg. (In Press)

Honors and Awards Relating to this Project:

For Dr. Martin D. Young:

Received the Jefferson Award from the South Carolina Academy of Science at its annual meeting in April of 1961 in recognition of his paper "Chloroquine Resistance in *Plasmodium falciparum*."

Visiting Lecturer and Temporary Advisor on Chemotherapy, PAHO Malaria Eradication Training Center, Kingston, Jamaica.

Member, Expert Advisory Panel on Malaria, World Health Organization

Serial No. NIAID-132-A
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A:

Project Title: Chemotherapy of Intestinal Parasites

Principal Investigator: Geoffrey M. Jeffery

Other Investigators: Andrew J. Harrison, Kenneth O. Phifer, and Martin D. Young

Cooperating Units: South Carolina State Hospital

Man Years (calendar year 1961):

Total	2 1/3
Professional:	1
Other:	1 1/3

Project Description:

Objectives:

To find adequate drugs for the treatment and prophylaxis of parasitic infections which constitute serious health problems in certain types of mental patients as well as in more normal populations; to investigate epidemiological factors relating to parasitism and its treatment and control; to determine how chemotherapeutic agents can be useful in the control of possible spread in institutionalized and non-institutionalized populations.

Methods Employed:

Various segments of institutional and normal populations are examined to determine qualitatively, and quantitatively when possible, their parasitic infections. Repeated examinations and long-term observations are frequently done to determine possible epidemiological factors of importance in a program of therapy. Promising compounds in varying dosages and regimens are tried against selected cases; post-treatment evaluation determines the effectivity of the drug against the various parasites. Repeated and long term treatments are done to eliminate parasite transmission in large, well-defined groups.

Major Findings:

Epidemiological studies in a defined institutional population were terminated after a final seventh year examination. Hookworm and Trichuris trichiura persisted in most patients for the entire period. Transmission of the protozoan parasites continued with some frequency during the early years of the study, but appeared to diminish significantly during the final three years.

Trials of higher dosages of bephenium chloride in three and five day regimens showed an increased effectiveness against Trichuris trichiura removing 68 and 88 per cent of the worms, respectively. The drug at these levels was highly effective against hookworm, but failure to eliminate Hymenolepis nana with this drug and with dichlorophen was noted.

Two population groups (a total of 213 persons) in a rural coastal area of South Carolina, investigated at the request of local and State health authorities, were found to be heavily infected with Ascaris lumbricoides (58 and 72 per cent) and T. trichiura (33 and 44 per cent). Both groups have been treated with piperazine (single doses of 3, 4, or 5 grams) for removal of Ascaris; results to date indicate 55 per cent cure and an overall worm reduction of 90 per cent. Blood studies prior to treatment revealed fairly normal hemoglobins, white and red cell counts, and hematocrits; elevation of eosinophiles in the two groups was severe, with average levels of about 15 and 20 per cent, respectively.

Significance to Bio-Medical Research and the Program of the Institute:

There continues to be great need for drugs adequate in the treatment and prevention of transmission of Trichuris, hookworm, and Ascaris in areas of high exposure risks. Better drugs, and better regimens of currently used drugs, are needed. Information obtained should be of particular value in areas where the control of these infections by sanitation is impossible or impracticable, such as in mental hospitals, economically retarded tropical or subtropical areas, and others.

Proposed Course of the Project:

The evaluation of promising compounds for better prophylactic and parasiticidal use will be continued with special emphasis on multi-purpose drugs, such as bephenium (Alcopara), new formulations of dithiazanine (Delvex), stilbazium iodide (Monopar), and others. New regimens of the older recognized drugs will also be investigated, particularly in an effort to control the worm infestation in populations not compatible with ordinary sanitation measures. This would include mental hospital groups and also groups similar to those being investigated in a nearby rural area. The use of piperazine at regular intervals will be attempted in the latter in an effort to control Ascaris infections in the absence of any sanitary improvements.

In addition to drug trials in humans, parallel investigations are underway on the mode of action of drugs on parasites. Efforts are being made to explain the failure of effective drugs to produce radical cures, the reduced effectiveness of drugs after initial treatment, and similar problems. Further investigations on the epidemiology of parasitic infections in mental hospitals and other groups will be continued.

Part B included - Yes.

Serial No. NIAID-132-A

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B.

Honors, Awards, and Publications.

Publications other than abstracts from this project:

Jeffery, G. M., Young, M. D., and Wyman, B. F., Jr. The Therapeutic and Prophylactic Effects of Dithiazanine and Tetrachloroethylene Against Worm Infections in Mental Patients. Am. J. Trop. Med. and Hyg. (In Press) 1961.

Serial No. NIAID-132-B
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Physiology of Parasites, with Special Emphasis on the Effect of Chemotherapeutic Agents

Principal Investigator: Kenneth O. Phifer

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 3/4
Professional: 3/4
Other:

Project Description:

Objectives:

To determine the place of action of drugs on parasites of man. The study of the action of drugs on these parasites includes the mechanism and locale, the development of resistance and a search for methods to avoid the development of resistance or how to overcome it.

Nucleic acids of malaria parasites are being examined qualitatively and quantitatively in order to determine whether drug-resistance affects the synthesis of these substances. To ascertain in what manner malaria parasites affect the mosquito's physiology. To determine what effect anthelmintics have on the *in vitro* metabolism and *in vivo* distribution of helminths.

Methods Employed:

Malaria-infected red blood cells are homogenized and fractionated into ribonucleic acid; this fraction is then analyzed chromatographically.

Enzymes in mosquito homogenates are characterized as to effects of substrate concentration, pH, temperature and time of incubation. Infected mosquitoes are compared with uninfected mosquitoes.

Helminths are incubated both in the presence and in the absence of anthelmintics, and several measures of metabolism are utilized: a general measure-rate of glucose uptake, a carbohydrate cycle enzyme assay-aldolase, amino acid cycle enzyme assays - transaminases, and a phosphatase assay. Also noted is the effect of these compounds on the distribution of the worms in the intestine of the host.

Major Findings:

Chromatographically, the acid-hydrolysate of ribonucleic acid from a chloroquine-resistant strain of Plasmodium falciparum separated into five components, whereas the chloroquine-susceptible material separated into only four.

Mosquitoes of the genera, Aedes, Anopheles, and Culex have been assayed for aldolase activity. For each genus, the effects of several variables have been noted: time of incubation, temperature, concentrations of substrate, pH and concentration of homogenate. These findings will serve as standards of reference for further studies with infected mosquitoes.

Bephenium chloride failed to reduce the numbers of Nippostrongylus muris in rats although its presence did bring about a relocation of worms from the anterior to the posterior part of the small intestine. Bephenium hydroxynaphthoate, on the other hand, reduced the number of worms by 50 per cent and also caused the worms to move posteriorly.

Tapeworms (Hymenolepis diminuta), exposed to atabrine in vivo, were not affected by this drug in vitro. The glucose uptake rates were not reduced in worms which had been exposed to the drug in vivo whereas rates of uptake were reduced in worms from rats which had not been given the drug.

Bephenium chloride, bephenium hydroxynaphthoate, atabrine and dichlorophen all reduced the glucose uptake of Hymenolepis diminuta and also inhibited p-nitrophenyl phosphatase activity. Both of these metabolic processes occur at the surface of the worm. On the other hand, several internal metabolic systems of this worm were not affected by these drugs. The drugs had no inhibiting effect on aldolase, aspartic acid-glutamic acid transaminase, nor alanine-glutamic acid transaminase.

Significance to Bio-Medical Research and the Program of the Institute:

Since several antimalarial agents have chemical structures similar to those of components of nucleic acids it seems advisable to determine whether these drugs affect the parasites' basic nuclear compounds. It is felt that if it were demonstrated that nucleic acids were altered by antimalarials, this clarification of mechanism of drug action would provide a more logical rationale for the synthesis of new drugs. Also,

demonstration of changes in nucleic acid composition in response to exposure to a drug should be of value in determining mechanisms of resistance.

Characterization of enzymes in uninfected and infected vectors of infectious disease may help clarify the relation of host and parasite and thus lead to a more rational approach to chemotherapy.

Study of the effect of anthelmintics on the distribution of helminths in the host intestine is important since in human cases of helminthiasis, a few worms often remain after therapy and the question of whether this is due to resistance to the drug or a relocation in the gut should be answered.

Study of the effect of anthelmintics on helminth metabolism will aid in a clearer understanding of drug action and might be valuable in the design of new drugs. The hypothesis that drugs act at the surface of tapeworms and are not necessarily taken through the surface of the tapeworm should be examined more thoroughly. The action of drugs on worms exposed to the drug in vivo may indicate whether or not some type of drug-resistance may arise.

Proposed Course of the Project:

Studies of nucleic acid composition of malaria parasites from drug-resistant and drug-susceptible strains will be continued using both paper chromatography and absorption spectrum analysis via spectrophotometry.

Comparisons will be made of the characteristics of enzyme systems of both infected and uninfected mosquitoes to determine the biochemical effects of parasitism on the host.

Animals infected with helminths will be drugged with anthelmintics and the worms will be counted and their position in the gut noted. Observations will be made of the action of the drugs against various in vitro metabolic assays. Studies on the location and mechanism of drug action in tapeworms will be extended.

Part B included : No.

Serial No. NIAID-132-C
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Virus-Parasite Associations

Principal Investigator: Geoffrey M. Jeffery

Other Investigators: William E. Collins

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1 1/2
Professional:	5/6
Other:	2/3

Project Description:

Objectives:

To determine experimentally if parasites might be associated with some of the virus diseases of man or animals as vectors or in some other capacity. To study such relationships under natural conditions and to investigate known virus infections and known populations to determine existing relationships, including the study of possible intestinal viruses among institutionalized mental patients. In view of current findings, further study the relationships of virus-malaria and virus-endoparasite associations under experimental conditions. Investigate the possibility that certain parasites may themselves be afflicted with harmful virus infections. Investigate the effects of parasitic infections upon virus infections and vice versa.

Methods Employed:

Small animals and their common parasites are used in attempts at transmission of viruses. Viruses, such as lymphocytic choriomeningitis (LCM), polio, St. Louis encephalitis (SLE), and encephalomyocarditis (EMC) are maintained in small animals by serial transmission, providing infective virus material for experimental work. Besides the common helminth parasites of mice, Nippostrongylus muris, Nematospiroides dubius, Hymenolepis nana, and others, the malaria parasites Plasmodium berghei and P. gallinaceum are also maintained as experimental parasites. Viruses and parasites are brought together in various ways, such as by

simultaneous infection in animals, during cultivation of the free-living stages, or by simultaneous exposure of the host to infective parasite stages and virus suspensions. Mosquitoes are dually infected with malaria parasites and viruses for transmission and infectivity studies. Tissue culture or small animals are used for detection of virus infection carried by parasites from virus-infected animals, for virus detection in parasites suspected of harboring viruses in nature, and for detection of virus infection in arthropods. Incidence of intestinal viruses and their possible association with parasites in institutionalized mental patients is studied with animal inoculation, tissue culture, and other means.

Major Findings:

In chicks inoculated with SLE virus and P. gallinaceum the appearance of viremia was somewhat earlier and for about the same duration as when virus was given alone. However, the viral titer appeared to be significantly lower in the dually infected chicks as compared with that in chicks receiving only virus.

Studies in EMC virus in chicks indicate that these hosts develop a viremia when inoculated, the titer decreasing with the increase of age in the chicks. It appears that the addition of P. gallinaceum to the system has a reducing effect on the quantity of circulating virus.

Confirmatory experiments have continued to verify the role of P. berghei in mice in the transport of SLE virus to the central nervous system when the latter is given intraperitoneally.

In preliminary studies on the dual infection of mice with N. muris and EMC virus, virus has been recoverable in good amounts from feces of all infected mice, regardless of the route of inoculation or the presence or absence of N. muris. The few experiments completed have not as yet revealed detectable quantities of virus incorporated in parasitic stages recovered and developed from virus infected mice. Exposure of N. muris larvae to high concentrations of EMC virus daily during cultivation did not produce infective larvae carrying detectable virus.

Significance to Bio-Medical Research and the Program of the Institute:

Many problems associated with the transmission of viruses in human and animal populations remain unsolved. New viruses in mammals and arthropods are continually being discovered. There are suggestions in the literature and theoretical possibilities that parasites may act as reservoirs and agents of transmission for viruses of importance to man. Elucidation of association of viruses and parasites, both experimental and natural, will do much toward bringing about a more complete understanding of the epidemiology of diseases produced by such organisms, alone or in combination. The possible role of malaria in transmitting

virus from host to host either by extending the viremia or by actually transporting the virus in the sporozoite is of epidemiological importance.

Proposed Course of the Project:

The general course of the project will be changed little. Repetition and expansion of the experiments combining nematode parasites and viral agents in the vertebrate host, during free-living stages, and in culture, will be carried out. An increased amount of effort will be directed toward the association of viruses and malaria parasites, and possibly other protozoans. The detection of viruses in malaria parasites, especially the sporozoites by fluorescent antibody techniques and by inoculation will be attempted. The possible significance of simultaneous infection of mosquitoes with virus and malaria parasites will be explored. Further refinement of viral detection methods will be investigated and incorporated in the work when practicable. Most of the experimental work planned is in the realm of artificial viral-parasite associations, but the possibility of extension of the studies to naturally occurring associations will be always in mind and such studies will be pursued as opportunities arise.

Part B included: Yes.

Serial No. NIAID-132-C
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B.

Honors, Awards, and Publications.

Honors Relating to this Project:

For Dr. Geoffrey M. Jeffery:

Elected Secretary-Treasurer of the American Society of Tropical Medicine and Hygiene.

Councilor for the South Carolina Academy of Science.

Training Grant for advanced studies in Virology and Public Health at the Yale University School of Medicine, September 1960 through May 1961.

Serial No. NIAID-132-D
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Development of Human Pathogens in Immature Insects

Principal Investigator: William E. Collins

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1
Professional:	1/3
Other:	2/3

Project Description:

Objectives:

To determine experimentally to what extent immature stages of arthropods will support growth of pathogenic organisms with particular emphasis on the viruses and protozoans in mosquito larvae. If experimental evidence indicates the growth or maintenance of these microorganisms in insect larvae, investigations will be made to determine the possible role larval infection plays in the transmission of these infections to man.

Methods Employed:

The larvae of mosquitoes belonging to several different genera are exposed to virus suspensions under varying conditions. The quantity of virus present in larvae, pupae, and adults subsequent to exposure is determined by small animal inoculations. Transmission of the virus by the adult mosquitoes is made using small animals and blood pools.

Major Findings:

Larvae of Aedes aegypti were exposed to mouse brain suspensions of St. Louis encephalitis virus (SLE). The virus was found in larval and pupal pools in detectable titers ranging from 2.5 to >6.0 mouse log IC LD_{50}. The minimum infection rates in pupae were at least two per cent. Adult male and female mosquitoes were shown to contain detectable

SLE virus titers up to 5.8 mouse log IC LD_{50}. Infection rates in adult mosquitoes were about one per cent. Nine confirmed transmissions of SLE virus by bite were obtained.

Larvae and pupae of *Culex pipiens quinquefasciatus* mosquitoes demonstrated SLE virus to a lesser degree than did *Aedes aegypti*. The infection rates in the adults were at least three per cent with 44 adult female and 11 adult male mosquito pools being positive for SLE virus. A total of 19 confirmed transmissions of SLE virus by larval exposed *C. pipiens quinquefasciatus* have been obtained.

Significance to Bio-Medical Research and the Program of the Institute:

The presence of viruses and protozoans in mosquito breeding pools as a result of improper sanitation procedures or by death and decomposition of infected animals and arthropods poses the possibility that these organisms may be taken up by the mosquito larvae and subsequently transmitted by the adults. Information on such larval infection will add to a more complete understanding of the epidemiology of a number of arthropod-borne diseases.

Proposed Course of the Project:

The general course of the project will be changed little. The ability of mosquito larvae of different genera to take up St. Louis encephalitis or other viruses and the effect of environmental conditions on the transmission of these infections will be tested.

Part B included: Yes.

Serial No. NIAID-132-D
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B.

Honors, Awards, and Publications.

Publications other than abstracts from this project:

Collins, Wm. E. Trans-stadial passage of St. Louis encephalitis virus in *Aedes aegypti* mosquitoes. Am. J. Trop. Med. and Hyg. (In Press) 1961.

Serial No. NIAID-132-E
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, South Carolina

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Insect Tissue Culture

Principal Investigator: William E. Collins

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):	
Total:	2/3
Professional:	1/3
Other:	1/3

Project Description:

Objectives:

To establish insect tissues in culture in order to better study those factors necessary for insect growth and metamorphosis and the effect of microorganisms on insect tissues. To determine which of the viruses, protozoans, or helminths will persist, develop or multiply in the different insect tissues grown *in vitro* using both natural vectors and unrelated species.

Methods Employed:

Tissues and cells from moth larvae and pupae and from mosquito larvae, pupae and adults are removed and set up in experimental culture media using several different culture methods. The presence of mitosis, tissue contractility or general cell condition are used as indications of tissue growth or maintenance. Viruses are added to such cultures and periodic samples collected and titrated in small animals. Protozoans are added and microscopic examinations made to determine persistence or development of the parasites. Guts of mosquitoes previously infected with *Plasmodium* sp. are removed and set up in culture. Periodic examination and measurement of the parasite development are made.

Major Findings:

A number of different lepidopteran tissues have undergone growth or extended maintenance to some extent in culture. Among these are caterpillar hemocytes and prepupal and pupal fatbody and cells of the ovariole sheath. The virus of St. Louis encephalitis has been maintained in cultures of caterpillar hemocytes from the catalpa sphinx (Ceratomia catalpae) for periods of up to 10 days. There appears to be no increase in virus titer in this tissue. Prepupal and pupal fat body cells readily proliferate in hanging drop culture remaining healthy for 10 to 14 days. Virus studies with SLE virus have indicated that the virus not only persists for an extended period in such cultures but appears to increase in titer between the sixth and eighth days of culture.

Significance to Bio-Medical Research and the Program of the Institute:

Insects serve as alternate hosts for a number of virus and protozoan diseases of man. Insect tissue culture would offer the opportunity to study these parasites and their growth in insect cells in vitro, and thus quite possibly shed new light on parasite growth requirements, arthropod tissue specificity and reasons why many human pathogenic viruses will multiply in the insect with no visible or apparent pathology. These studies may yield information on those factors controlling the ability of insect vectors to transmit virus and protozoan diseases whereas closely related species of insects do not.

Proposed Course of the Project:

Various tissues of mosquito larvae, pupae, and adults and moth larvae and pupae will be established in culture and tested for their ability to support the virus of St. Louis encephalitis. Microscopic studies will be made in efforts to detect any cytopathology of the tissue as a result of virus infection. In addition, fluorescent antibody studies will be made to determine in which cells or tissues the virus is present. Guts from mosquitoes infected with Plasmodium sp. will be set up in different culture media in an attempt to determine those factors necessary for the development of sporozoites in vitro. Further studies on the effect of virus on caterpillar blood cells and ovariole tissue will be made. Further studies on the effect of virus on lepidopteran prepupal and pupal fatbody and ovarial tissue will be made.

Part B included: No.

Serial No. NIAID-133
1. Parasite Chemotherapy
2. Cytology
3. Chamblee, Georgia

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Studies of the biology of simian malaria and of human malaria in simians

Principal Investigators: Charles G. Dobrovolny and Don E. Eyles

Other Investigators: Robert L. Ingram, Peter Contacos, Harvey Elder, Frances E. Jones and John R. Jumper

Cooperating Units: Section on Clinical Studies, Laboratory of Parasite Chemotherapy
Communicable Disease Center, Atlanta, Georgia

Man Years (calendar year 1961):

Total:	5
Professional:	3
Other:	2

Project Description:

Objectives: 1) To determine and describe the stages in the life cycle of newly isolated species and/or strains of simian malaria with special emphasis on the development of exoerythrocytic bodies; compare the morphology and the host parasite relationship of the isolates with known species; to provide materials and correlative information for projects on development of exoerythrocytic parasites *in vitro* and the chemotherapy of human malaria. 2) Employing various methods of inoculation to determine if any of the human malarias can be grown in simians; determine the types of cells parasitized, and trace the development from sporozoite to mature schizont.

Methods Employed: 1) New isolates of monkey malaria received from the Far East Research Unit and other sources are maintained in monkeys or held in the deep freeze. Transmission from host to host is accomplished through intravenous inoculation of infected blood. For study of exoerythrocytic stages transmission is made by intravenous inoculation of large numbers of salivary glands dissected from infected mosquitoes. Liver biopsies made on selected days after inoculation of monkeys with sporozoites are examined microscopically for exoerythrocytic bodies. 2) The same techniques are employed in dealing with human malaria in monkeys.

Major Findings: A new species of vivax-type malaria isolated from the pigtailed macaque (*Macaca nemestrina*) from Malaya was described. This parasite was passed to other species of macaques and was also studied in normal and splenectomized rhesus monkeys. It was named *Plasmodium fieldi*.

Isolates of new strains of *P. cynomolgi*, *P. inui* and *P. knowlesi* from Southeast Asian monkeys were studied. Some of these isolates like *P. fieldi* may also prove to be new species.

Five species of Haematozoa were recovered from the livers and blood of *Macaca nemestrina* the pigtailed monkey from Malaya. Attempts at passage to other species of monkeys were not successful.

Exoerythrocytic stages of *P. inui* (characteristics similar to human *P. malariae*) and *P. gonderi* (vivax type) were studied. Attempts to establish these simian malaria strains in man are referred to under Project No. 134.

Rhesus monkeys inoculated with human blood infected with *P. vivax* as well as those that received blood infected with *P. falciparum* failed to develop parasitemias. Biopsies taken from a number of monkeys inoculated with sporozoites of *P. vivax* were examined for exoerythrocytic parasites. The results are inconclusive.

This project was curtailed due to failure to raise and maintain colonies of suitable mosquito hosts for an extended period of time. Also much time was spent in relocation of the laboratory from Memphis to Chamblee.

Significance to Bio-medical Research and the Program of the Institute: This project is part of an intergraded program of the Laboratory of Parasite Chemotherapy to study simian malaria in monkeys and man. Studies are made of the biology (host-parasite relationship, life cycles, etc.) of the species and strains of simian malaria in monkeys in order to proceed with chemotherapeutic studies and studies of the parasites in man. Any degree of success in the passage of human malaria to monkeys would be a worth-while scientific contribution in making available a lower animal host for study of the disease and its possible control.

Proposed Course of the Project: The studies will be continued and extended to include investigations of new species and strains of simian malaria from monkeys and apes from the old as well as the new world. Chemotherapeutic studies for evaluation of antimalaria drugs, not conducted during the past year, will be resumed. Priority will be given to studies of species, such as *P. inui*, which have not been established in man. Also on the agenda for next year are: a search for more efficient insect vectors of monkey malaria; passage of simian malaria to other species of primates; resumption of attempts to grow the parasites in tissue cultures and immunological studies.

Part B included - Yes

Serial No. NIAID-133

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Eyles, D. E. Susceptibility of *Anopheles albimanus* to primate and avian malarias. Mosquito News, 20:368-371, 1961.

Eyles, D. E. and Coatney, G. R. Effect of certain drugs on exoerythrocytic parasites of *Plasmodium cynomolgi*. (In press)

Eyles, D. E. The Experimental Chemotherapy of Toxoplasmosis. "Experimental Chemotherapy" by Schnitzer and Hawking. (In press)

Eyles, D. E. Chemotherapy of Toxoplasmosis. Proceedings of Council for Research in Glaucoma and Allied Diseases. (In press)

Serial No. NIAID-133-A
1. Parasite Chemotherapy
2. Cytology
3. Chamblee, Georgia

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Immunological studies of malaria parasites.

Principal Investigator: Robert L. Ingram

Other Investigators: Charles G. Dobrovolny and John R. Jumper

Cooperating Units: None

Man Years (calendar year 1961):

Total:	3/8
Professional:	1/4
Other:	1/8

Project Description:

Objectives: To study immunological relationships among simian malarias and between simian malaria and certain species of human malarias. Also, to study the immunological similarity between different stages of the same malaria species.

Methods Employed: Antibodies were obtained from convalescent animals which had been infected several times with a species of malaria and challenged with the homologous species until no parasitemia developed. Antibodies were also obtained by injecting rabbits with infected blood from monkeys. Sporozoites were injected into rabbits as another way of obtaining antibodies. Serum was collected from the animals and fractionated with ammonium sulfate to obtain the gamma-globulin component; this fraction was dialyzed against buffered saline, quantitated for the amount of protein, and conjugated with fluorescein isothiocynate (F.I.C.). This preparation was used in the fluorescent antibody studies in a conventional way. Tissue was removed from monkeys by laparotomy and prepared according to standard histological techniques.

Major Findings: Serum obtained from hyperimmune monkeys which had been infected with the blood forms of the B strain of Plasmodium cynomolgi readily stained the blood stages of the homologous species by the fluorescent antibody technique. Blood stages of Plasmodium cynomolgi, Plasmodium gonderi, and Plasmodium inui were also stained with the labeled B strain serum. The degree of the interaction was difficult to determine by the fluorescent antibody method. Later, attempts were made to stain the human malaria species, P. vivax, P. malariae, and P. falciparum with the B strain serum by the

fluorescent antibody technique. None of these parasites showed an appreciable reaction.

Significance to Bio-medical Research and Program of the Institute: The demonstration that convalescent serum from a malaria infected animal can be used to react with the homologous parasite, and in some cases a hertero-logous species of malaria, by the fluorescent antibody method provides a new tool for the epidemiologist in the study of malaria. It should be possible now to tell not only if the individual has malaria, but whether or not he has had it in the immediate past. The finding has facilitated the use of the fluorescent antibody technique in the study of the cycle of simian malaria in the mammalian host. When the fluorescent antibody technique becomes sufficiently quantitative, its application to the study of malaria as a zoonosis should have some utility.

Proposed Course of the Project: Studies of the immunological interaction between simian and human malarias will be extended. Further studies of the immunological relationships among simian malarias will be correlated with morphological, physiological, and host susceptibility investigations.

Part B included - Yes

Serial No. NIAID-133-A

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Ingram, Robert L., Otken, Luther B., Jr. and Jumper, John R. Staining of malarial parasites by the fluorescent antibody technic. Proc. Soc. Exp. Biol. and Med., 106: 52-54. 1961.

Honors and awards relating to this project:

None

Serial No. NIAID-133-B
1. Parasite Chemotherapy
2. Cytology
3. Chamblee, Georgia

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Tissue and cell cultures.

Principal Investigator: Robert L. Ingram

Other Investigators: Frances E. Jones and John R. Jumper

Cooperating Units: None

Man Years (calendar year 1961):

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objective: To develop methods for maintaining adult liver tissue with a view of studying the tissue phases of malaria parasites *in vitro*. These studies to include biology of the organism, effects of chemotherapeutic agents against the parasite, and biochemical studies of host-parasite relationships.

Methods Employed: Adult liver tissue is obtained from monkeys by laparotomy; other tissues are taken from chick embryos; monkey kidney and other cells are cultured as monolayers. Standard tissue culture methods are employed. Various compositions of cell nutrient materials are tested for their effect upon the tissue, and hormones and cofactors are added to the medium; the conditions are varied to give the most favorable results. Cytochemical, histological, and cell count methods are used to evaluate the success of the cultures.

Major Findings: Sufficient success has been obtained in maintaining adult organized monkey liver cultures that many problems of the exoerythrocytic phase of simian malaria can be studied *in vitro*. Excellent survival of the tissue in culture for 2 days, and survival of 50% or more of the tissue for 4 days has been accomplished. A number of variations of the composition of the culture medium and accessory factors, which were reported previously, has been made; none have improved substantially the survival time of the tissue. However, the additional tests have given further support to the previous findings.

Most of the cofactors, hormones and other compounds which were used in the adult liver tissue experiments, were tested for their effect on monkey kidney cells cultured in roller tubes. The effects of cortisone, insulin, growth hormone, sulfapyrazine, pyrimethamine, 6-mercaptopurine, and aminopterin in these cultures were comparable to those that were produced when these compounds were added to the medium of the adult liver cultures. A possible exception being that growth hormone appeared to stimulate cell proliferation, whereas it apparently had no effect of increasing the survival of the adult tissue.

Other findings indicate that drugs which are used as chemotherapeutic agents may interfer with the survival of the tissue in which the parasites are grown and, therefore, make difficult the evaluation of the drug against the organism.

Significance to Bio-medical Research and the Program of the Institute: Adult liver tissue apparently has been one of the most difficult tissue to maintain in vitro. Consequently, any success along these lines would be of interest to tissue culture workers. Since the liver performs so many functions, successful culture in vitro would make many studies possible. In addition, many parasitic organisms have a part of their cycle in the liver. It would be useful to study the liver stages of these organisms in vitro. In certain biochemical investigations the use of intact tissue would be more desirable than homogenates or the usual tissue slices as the tissue could be studied for a much longer time.

Proposed Course of the Project: The methods developed from these investigations will be utilized to study the tissue stages of simian malarias. These studies will include biology of the parasite, chemotherapeutic agents, and elucidation of the malaria cycle in the monkey.

Part B. included - No

Serial No. NIAID-133-C
1. Parasite Chemotherapy
2. Cytology
3. Chamblee, Georgia

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Biochemical studies of simian malarias.

Principal Investigator: Robert L. Ingram

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 5/8
Professional: 1/2
Other: 1/8

Project Description:

Objectives: To study the biochemistry of malaria organisms and to utilize this information to further the study of chemotherapeutic agents against the parasite. Also, to determine the cycle of the organism in the mammalian host by biochemical methods.

Methods Employed: A number of simian malarias, along with *Plasmodium gallinaceum*, and *Plasmodium berghei*, are maintained in the laboratory, and constitute the source material for these studies. In general, standard biochemical procedures are followed for the isolation, separation, and identification of the various chemical components being studied. Hot trichloroacetic acid (TCA), perchloric acid (PA), or 10% sodium chloride is used to extract nucleic acids from the organisms. Following some preliminary treatments, the nucleic acids are precipitated with 2.5 volumes of cold ethanol. The nucleic acids are separated into deoxyribonucleic acid (DNA) and nucleotides of ribonucleic acid (RNA). DNA is hydrolyzed with hot HCL or perchloric acid. Chromatographic and spectrophotometric methods are used to isolate and/or identify the various components of the hydrolysis mixtures. Suitable substrates are used when enzymes are studied.

In the case of the isotope tracer studies, infected mosquitoes are fed H^3-labeled thymidine; sporozoites from these mosquitoes are injected into a "clean" monkey. Tissue is taken from this animal for histological sections; autoradiographs of these sections are made by standard procedures.

Major Findings: Nucleotides and nucleic acids have been extracted from a number of malaria species. Most of the common mononucleotides of adenosine, guanosine, cytidine, and uridine have been tentatively identified from the cold trichloroacetic acid or acid soluble fraction. Nucleotides of adenosine are present in greatest quantity and those of cytidine the least. Compounds of inosine (hypoxanthine riboside) are always present, but have not been more specifically identified. Thus far, of the malarias studied, all appear to have essentially the same nucleotides in the acid soluble fraction. The parasites contain predominately deoxyribonucleic acid (DNA). The amount of DNA present in any infected blood sample is proportioned to the number of parasites present. The amount of ribonucleic acid (RNA) decreases as the number of parasites increase.

Results of the study of the cycle of the B strain of P. cynomolgi in the monkey by means of isotope tracer and the fluorescent antibody techniques are inconclusive. This work was greatly curtailed by the lack of mosquitoes following the move from Memphis.

Significance to Bio-medical Research and the Program of the Institute: As nucleic acids play such an important role, not only in the nature of the organism, but also in its survival, information concerning these biologically important compounds from any source is likely to be useful. Also, information of this kind is noticeably lacking from parasitic protozoa and other parasitological organisms. Information gained from these studies should have application in our chemotherapeutic research and possibly that of other workers concerned with similar problems.

The isotope tracer method employing autoradiographs has wide application in biochemistry and the successful utilization of this technique to solve parasitological problems should prove useful to other biologists.

Proposed Course of the Project: Studies of the nucleic acids and their components will be continued. Numerous samples of DNA, and nucleotides of RNA from many different malaria organisms are on hand. These fractions await further studies. *In vitro* cultures or suspensions of the blood forms of the organisms will be tested for various enzyme systems. The effect of antimetabolites and other compounds on these systems will be determined. These studies also will be conducted on the e.e. stages of malaria.

Part B included - No

Serial No. NIAID-134
1. Parasite Chemotherapy
2. Section on Clinical Studies
3. Atlanta, Georgia

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Studies on simian malarias in human volunteers

Principal Investigator: G. Robert Coatney

Other Investigators: Peter G. Contacos, Harvey A. Elder, Charles G. Dobrovolny, and Don E. Eyles

Cooperating Units: Department of Justice, Bureau of Prisons, and Institute for Medical Research, Christ Hospital, Cincinnati, Ohio

Man Years (calendar year 1961):

Total:	6
Professional:	1 1/3
Other:	4 2/3

Project Description:

Objectives:

To continue the study of simian malarias in human volunteers with special emphasis on transmissibility from man to man by mosquitoes.

Methods Employed: Normal inmate volunteers at the U. S. Penitentiary, Atlanta, Georgia, are screened before they are admitted to the project. Volunteers are under the care of the resident investigators whether handled as outpatients or as inpatients. They are exposed to infection either by the bites of infected mosquitoes or by the intravenous inoculation of parasitized homologous blood. They freely submit themselves to all necessary laboratory procedures and receive medications only as prescribed. Participation on a project varies from 6 to 18 months depending on the nature of the project.

Major Findings:

Plasmodium cynomolgi bastianellii (hereafter referred to as the B strain of P. cynomolgi) and Plasmodium cynomolgi cynomolgi (hereafter referred to as the M strain of P. cynomolgi)

The implications of the establishment of 2 strains of P. cynomolgi in man, especially as they may relate to malaria eradication, gave top priority to our studies with simian malarias in man during calendar year 1961.

As soon as we obtained a human infection with the M strain of *P. cynomolgi* following bites of mosquitoes infected from a monkey, it seemed desirable to determine whether the capacity of this strain to produce infections in man could be potentiated by serial passage. The infection was transferred by blood from the original human infection to a clean volunteer and then to a monkey. *A. freeborni* mosquitoes, infected by feeding on this monkey, were allowed to bite 4 volunteers. Three of these 4 volunteers developed patent infections in 17 to 19 days, in contrast to only one patent infection in 82 days in the original eleven transmission attempts. This strain was also passaged serially by blood inoculation from the original human infection through 3 human volunteers. Maximum parasitemias in the 1st, 2nd, and 3rd passages were 160, 1250, and 8300 parasites per cmm. of blood, respectively. These results suggest that once established in man, serial passage of this strain not only potentiated its transmissibility from monkey to man by mosquitoes, but also its ability to adapt to the human being as a host.

Life pattern (relapse) studies using the B strain of *P. cynomolgi* are in progress. In the 7 volunteers who developed patent infections, no unequivocable relapses have occurred up to 130 days after treatment on one day with 30 grains of quinine sulfate.

Infections produced by the inoculation of blood parasitized with the B or M strain of *P. cynomolgi* exhibited no significant differences in clinical manifestations. However, when infections induced by mosquito bites are compared, several differences were apparent: the duration and height of the fevers were greater, the tertian patterns were more numerous and typical, and the frequency of splenomegaly was higher in volunteers infected with the M strain than in those infected with the B strain.

Twelve attempts at man to man transmission by the bites of *Anopheles freeborni* infected with *P. cynomolgi*, 7 with the M strain and 5 with the B strain, were successful. These results suggest that normal, i.e. mosquito, transmission of these 2 strains of simian malaria can occur from man to man with a high degree of frequency. It was also demonstrated that simian malaria can be transmitted from man back to the monkey by mosquitoes infected from man. We consider it even more significant to have been able to transmit simian malaria consecutively from monkey to man to man to man by mosquito bites. In this connection, it should be noted that mosquitoes can become infected in the presence of very low gametocyte counts in human beings infected with simian malaria (less than 50 gametocytes per cmm. of blood), and further, that relatively few bites of these infected mosquitoes will produce the full syndrome of malaria in clean volunteers.

Plasmodium inui and *Plasmodium gonderi*

One attempt to transmit *P. inui* (a quartan-like parasite) and 3 attempts to transmit *P. gonderi* (a vivax-like parasite) from monkey to man by sporozoites have been unsuccessful to date.

Significance to Bio-medical Research and the Program of the Institute: This country, along with other countries of the world, is committed to a program of world-wide eradication of malaria. In the past, it has been considered by most malariologists that non-human reservoirs would not be a problem in malaria eradication. However, the results of these studies suggest that we should reconsider our thoughts on the role of non-human reservoirs of malaria in programming for world-wide eradication of malaria.

Proposed Course of the Project: To continue studies on simian malarias with emphasis on the following: (1) the establishment of other simian species of malaria in man, (2) in vivo cross immunity studies between simian and human malarias, and (3) increasing the ability of simian malarias to adapt to human beings by the rapid passage of parasites through up to 20 volunteers.

Part B included - Yes

Serial No. NIAID-134

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Beye, H.K. Getz, M.E., Coatney, G.R., Elder, H.A., and Eyles, D.E. Simian malaria in man. Am. J. Trop. Med. and Hyg., 10:311-316, 1961.

Coatney, G.R., Elder, H.A., Contacos, P.G., Getz, M.E., Greenland, R., Rossan, R.N., and Schmidt, L.H. Transmission of the M strain of *Plasmodium cynomolgi* to man. Am. J. Trop. Med. and Hyg., 10:673-678, 1961.

Contacos, P.G., Elder, H.A., Coatney, G.R. and Genther, C. Man to man transfer of two strains of *Plasmodium cynomolgi* by mosquito bite. Am. J. Trop. Med. and Hyg., in press.

Kuvin, S.F., Beye, H.M., Stohlman, F., Jr., Contacos, P.G. and Coatney, G.R. Clinical and physiological responses in sporozoite-induced *Plasmodium cynomolgi bastianellii* and *Plasmodium vivax* infections in normal volunteers. J. Clin. Invest., in press.

Serial No. NIAID-134-A
1. Parasite Chemotherapy
2. Section on Clinical Studies
3. Atlanta, Georgia

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Studies in human malaria

Principal Investigator: G. Robert Coatney

Other Investigators: Peter G. Contacos, Harvey A. Elder, Martin D. Young, and Geoffrey M. Jeffery

Cooperating Units: Department of Justice, Bureau of Prisons

Man Years (calendar year 1961):

Total:	2 1/3
Professional:	2/3
Other:	1 2/3

Project Description:

Objectives: To evaluate new antimalarial drugs and to study parasite resistance to drugs as they may relate to malaria eradication.

Methods Employed: Normal inmate volunteers at the U. S. Penitentiary, Atlanta, Georgia, are screened before they are admitted to the project. Volunteers are under the care of the resident investigators. They are exposed to infection either by the bites of infected mosquitoes or by the intravenous injection of parasitized blood. They submit freely to necessary laboratory procedures and receive medications only as prescribed. Participation may be from 6 to 18 months depending on the nature of the project.

Major Findings:

Burroughs-Wellcome 377C54:

B-W 377C54, 1,6-dihydroxy-2,5-bis(cyclohexylaminomethyl) naphthalene, has been reported to be a good schizontocide against human malarias, with activity similar to that of chloroquine. Since this drug had not been tried in this country, we used it in 3 cases of falciparum malaria. The drug in each case was given at a dosage schedule twice that used in studies reported in the available literature. It was administered as follows: 300 mg. orally every 12 hours for 4 doses, resulting in a total dosage of 1.2 grams given in 36 hours. The first case was an infection with a South Rhodesian strain of P. falciparum. Before treatment, the count was 13,000 parasites per cmm of blood. Twelve hours after the last dose was given, blood smears were

negative. At 5 days post-therapy, gametocytes appeared; by the 16th day, asexual stages reappeared. The patient was retreated with the same drug regimen. Twenty-seven days after the 2nd treatment, parasites again reappeared. The infection was finally terminated with 600 mg chloroquine base given orally in one dose.

The other 2 cases were patients with infections of a chloroquine resistant Colombian strain of P. falciparum that broke through mepacrine suppression. In one case, 377C54 therapy was started when the parasite count was 2,500 per cmm. of blood. The counts were 25,000 at 12 hours and 7,500 at 36 hours when the last dose was given. At 48, 60, and 70 hours, the counts were 10,000, 12,500, and 25,000 parasites per cmm., respectively. The other case responded in a similar manner although parasitemia was of a lower order. The parasites were cleared from the blood temporarily by 120 grains of quinine sulfate. Our experience with this drug (although limited) would suggest that it is not a curative antimalarial for P. falciparum in the dosage regimen used.

Colombian strain of Plasmodium falciparum

This project was initiated to determine whether true cross-resistance to mepacrine exists in this strain of P. falciparum resistant to 4-aminoquinolines. Two volunteers were put on suppressive mepacrine, 100 mg. daily for 6 days each week. Both had been on suppressive therapy for at least one week before exposure to infection by mosquitoes. Each volunteer had a bite partner to serve as a control. One volunteer on mepacrine suppression and his control developed patent infections in 12 days. The other volunteer on suppression developed a patent infection in 13 days; his control (a Negro) has not exhibited patency of infection. The one infected control was started on the therapeutic regimen of mepacrine (2.8 grams in 7 days) on the 4th day of parasitemia (6,000 parasites per cmm. of blood). In 24 hours, he had received the loading dose of one gram of mepacrine. At 36 hours and 72 hours, the parasite counts were 35,000 and 60,000, respectively. Since the parasitemia continued to increase rapidly even after 3 days of mepacrine therapy, quinine sulfate was initiated at a dosage of 30 grains daily for 5 days. Although the parasites were removed in 7 days, they reappeared 7 days after quinine therapy.

Venezuelan strain of Plasmodium vivax

Basic life pattern (relapse) studies are in progress with this strain of P. vivax. Seven volunteers developed patent infections with this strain following exposure by mosquito bites. After 3 days of parasitemia and one febrile episode of 101°F. or higher, they were treated with a total of 150 grains of quinine sulfate given over a period of 5 days. Since the completion of therapy, they have been followed for from 122 to 169 days. Five of the 7 volunteers have relapsed once, ranging from 88 to 161 days post-therapy.

Significance to Bio-medical Research and the Program of the Institute: Studies on human malaria in volunteers are significant as they relate to malaria eradication. The knowledge obtained from evaluating new antimalarials, singly or in combinations, and under well-controlled conditions, could be applied in the field of malaria eradication programming. The importance of parasite resistance to known effective antimalarials, especially in terms of malaria eradication is obvious.

Proposed Course of the Project: To continue studies on drug evaluation (several new antimalarial preparations) and parasite resistance in terms of malaria eradication and immunity studies with human malaria.

Part B included - Yes

Serial No. NIAID-134-A

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Coatney, G. R. and Getz, M. E. Primaquine and quinocide as curative agents against sporozoite-induced Chesson strain vivax malaria. Bull. Wld. Hlth Org., in press.

Kuvin, S.F., Tobie, J.E., Evans, C.B., Coatney, G.R. and Contacos, P.G. Antibody production in human malaria as determined by the fluorescent antibody technique. Science, in press.

Serial No. NIAID-135
1. Parasite Chemotherapy
2. Far East Research Unit
3. Kuala Lumpur, Malaya

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Studies of the vectors of simian and human malarias.

Principal Investigators: Don E. Eyles, R. H. Wharton, McWilson Warren

Other Investigators: None

Cooperating Units: Institute for Medical Research, Federation of Malaya (Division of Entomology)

Man Years (calendar year 1961):

Total:	3
Professional:	1
Other:	2

Project Description:

Objectives: To study the transmission of simian malaria as related to human malaria; 1. To determine the species of mosquitoes which act as vectors of simian malaria; 2. To determine if these are the same as the vectors of human malaria; 3. To study the feeding habits of the simian vectors, comparative biting rates in canopy and on ground, and frequency with which man is attacked; 4. To determine the origin of the sporozoites found frequently in some oriental *Anopheles* which are of doubtful primate origin; 5. To determine experimentally the susceptibility of common oriental *Anopheles* to infection with strains of simian malaria.

Methods Employed: Mosquitoes are systematically collected and dissected. Sporozoites found are injected intravenously into uninfected rhesus monkeys and the monkeys observed for infection. The mosquito fauna and simian/human malaria prevalence is studied in several ecologically distinct areas (i.e., coastal mangrove border, lowland swamp forest, foothills, high jungle.) Platforms are erected in the canopy, and systematic trapping using monkey and human bait conducted at intervals over an extended period. Searches are made for freshly engorged mosquitoes and tests run to determine origin of blood meals. Sentinel monkeys are exposed to risk of malaria infection in selected situations. A variety of species of mosquitoes are kept in the insectary through rearing from eggs deposited by wild caught females and the progeny fed upon infected monkeys to determine degree of susceptibility. Wild caught mosquitoes are also used for the same purpose. Efforts are made to establish insectary colonies.

Major Findings: It has been found that Anopheles hackeri is a vector of simian malaria. Seven sporozoite infections were found and the glands injected into monkeys, six producing infection. Three of the infections were Plasmodium knowlesi; the other three were of a tertian type but differing apparently from both P. cynomolgi and P. fieldi. Morphological studies continue of these strains. Anopheles hackeri, once believed to be a rare mosquito, breeds in Nipah palm bases and fallen bamboos, particularly along the mangrove fringe on the west coast.

It has been found that A. hackeri bites monkeys in the canopy more frequently than on the ground, but occasional hackeri adults are taken in human bait traps at ground level.

Precipitin test work indicates this species feeds on primates but more refined methods may have to be used to determine species. Similar trapping information indicates A. maculatus bites in the canopy but frequently on the ground; A. sundaicus almost exclusively on the ground; A. leucosphyrus most readily in the canopy but frequently at ground level and is attracted to both man and monkey. A. umbrosus and letifer bit most readily on the ground, and A. umbrosus has an extremely high prevalence of sporozoites of unknown origin, which are neither human or macaque malaria.

Experimentally it has been shown that A. maculatus, A. sundaicus, A. philippinensis, A. kochi, and A. hackeri are susceptible, in a moderate to high degree, to infection with Plasmodium cynomolgi. A. vagus, A. barbirostris (light wing), A. sinensis, A. pediteeniatus, A. indiensis, and A. letifer are insusceptible or of low susceptibility. Samples upon which these conclusions are drawn are small and therefore subject to modification.

Significance to Bio-medical Research and the Program of the Institute: Although it has been shown in the laboratory that potentially simian malaria is transmissible readily to man, the likelihood of this happening in nature is largely dependent upon the habits of the vectors. Prior to this project no simian malaria vector had been identified with certainty. Completion of the project will allow an estimate of the zoonosis hazard. In addition, studies of the epidemiology of simian malaria may yield useful information applicable to epidemiology of the disease in man. Finally, this study has called attention to the fact that too much weight must not be placed on the results of dissections of field-caught mosquitoes, as sporozoites may be of human, simian, or non-primate origin.

Proposed Course of Project: The course of the project will take the following lines: 1) Complete studies of the habits of potential vectors in selected ecological communities have resulted in the identification of one vector to date. 2) Select additional areas for study where information of significance may be obtained, with the view of determining the importance of simian malaria vectors. 3) Continue comparative study of biting habits of the important Anopheles. 4) Continue studies to discover the origin of sporozoites in certain mosquitoes which are probably of non-primate origin. 5) Continue

studies of the susceptibility of oriental human malaria work and extending the human malaria work if necessary. 6) Study, in particular, the mosquitoes of the Anopheles leucosphyrus group (including balabacensis) which are present in areas where malaria eradication has not succeeded and which are believed to bite both monkeys and man readily.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Wharton, R.H. and Eyles, D.E. Anopheles hackeri, a vector of Plasmodium knowlesi in Malaya. Science, 134:279-280. 1961

Serial No. NIAID-135-A
1. Parasite Chemotherapy
2. Far East Research Unit
3. Kuala Lumpur, Malaya

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Study of cross transmission of simian malaria to man under field conditions.

Principal Investigators: Don E. Eyles, McWilson Warren

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: It has been amply demonstrated in the laboratory not only that certain simian malarias are readily transmissible to man but that the malarias can be transmitted from man to man. This can be accomplished by means of blood inoculation, but more significant, it can be done by mosquito passage also.

The objective of the present project is to determine if such transmission occurs under field conditions. If so, it must be determined the frequency with which this takes place.

Methods Employed: Malaria studies are made in aborigines and others, particularly of groups living in close contact with monkeys. Blood from selected persons, particularly those with low grade vivax-type parasitemias, is taken and inoculated into uninfected rhesus monkeys. Monkeys are splenectomized to increase susceptibility either before inoculation or after, and are observed for the appearance of parasitemia.

In countries in which malaria eradication is in progress, situations are examined in which malaria continues to persist in the face of eradication efforts. Arrangements will be made with the appropriate Public Health Officials to study such situations cooperatively using procedures similar to the preceeding, but including, as well, correlated studies of monkeys and mosquitoes in the study areas.

Serial No. NIAID-135-A

Major Findings: No positive findings have come out of this study as yet. A total of 35 blood specimens primarily from aborigines have been injected into rhesus monkeys without result. Since cross transmission, if it occurs, may be rare and local in occurrence, it is considered that a much larger sample will be necessary before conclusions can be made from the negative findings.

Significance to Bio-medical Research and the Program of the Institute: Should it be found that man in the orient is infected, even though rarely, with malarias transmissible from monkeys, it will be of the utmost significance to the worldwide malaria eradication effort. Positive findings will require a reorientation of the philosophy of eradication, and a new research approach to the problem of eradication. The program of the Institute, in addition to study of fundamental problems, is to some extent directed toward problems with application to malaria eradication, and reorientation will also be necessary should it be demonstrated that malaria is a zoonotic disease.

Should it become evident that malaria is not a zoonotic disease in the field, it is equally important to existing programs that this be demonstrated.

Proposed Course of Project: During the next year, arrangements will be made with the Ministries of countries adjacent and near Malaya in which eradication is being attempted to cooperate in the study of foci of persistent malaria. Within Malaya the search for possible field cross-transmission will be continued.

Part B included - No

Serial No. NIAID-135-B
1. Parasite Chemotherapy
2. Far East Research Unit
3. Kuala Lumpur, Malaya

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A

Project Title: Systematic studies of the malaria parasites of monkeys and apes of Malaya and nearby territories.

Principal Investigators: Don E. Eyles, McWilson Warren

Other Investigators: Elizabeth Guinn, Clinton S. Smith

Cooperating Units: Institute for Medical Research, Federation of Malaya (Divisions of Entomology and Malariology)

Man Years (calendar year 1961):
Total: 4
Professional: 1
Other: 3

Project Description:

Objectives: To determine the types of malaria found in Asian monkeys and apes, describing them systematically and isolating certain strains for trial in man (see NIAID-133). To develop objective methods for morphological study of malaria parasites, comparing newly recovered parasites with those previously described, and with those of man.

Methods Employed: Monkeys and apes are procured from a variety of sources: purchase from commercial animal trappers; purchase from aborigines; shooting and trapping by PHS personnel; through cooperation with Game Department and other governmental agencies. Initial examination is made of blood films and data on species, sex, wt., etc. for a determination of over all prevalence. Monkeys with patent infections and others with no apparent infection are studied following splenectomy and subinoculation of infected, or, possibly infected, blood into uninfected rhesus monkeys. Strains of malaria recovered are studied morphologically to see if they can be relegated to known species. Certain strains are intensively studied to determine significance of differences noted. Most strains are preserved by freezing for possible future use, and some are transmitted to the United States for study in man. Cross-specific transfers are made, especially to Macaca irus monkeys reared in the laboratory and thus known to be malaria free.

Patterns of parasitemia and morphological characteristics in the different hosts are compared.

Major Findings: It has been found that approximately 33 per cent of pig-tail macaques (Macaca nemestrina) and 40 per cent of long-tail macaques (Macaca irus) of Malaya are infected with malaria. Hepatocystis semnopitheci was discovered for the first time in M. nemestrina. A new species of malaria was found in this macaque also and is to be designated Plasmodium fieldi in honor of Dr. John Field, former director of the Institute for Medical Research in Kuala Lumpur. Plasmodium knowlesi and P. inui were also found in the Malayan macaques.

The presence of malaria parasites was also demonstrated in M. irus and M. nemestrina leonina from Thailand and the latter monkey from East Pakistan. Plasmodium knowlesi and P. inui as well as a species of Hepatocystis were found in M. irus laeta on an isolated island off the east coast of Malaya.

Leaf monkeys of the genus Presbytis were studied in Malaya. A significant proportion of all three Malayan species were found infected with malaria (P. cristatus, obscurus, and melalophos) and the parasites are now being studied.

The finding of malaria infected rhesus monkeys from East Pakistan by Schmidt has been confirmed and two localities from which infected monkeys came were pinpointed.

Significance to Bio-medical Research and the Program of the Institute: The possibility of malaria as a zoonosis could be of extreme importance in malaria eradication. The present investigation is fundamental in evaluating the zoonosis possibilities. Integrated with studies in human volunteers, the general properties of monkey malarias as related to transmissibility to man will be disclosed.

Proposed Course of the Project: Study of the Malayan species of monkeys and their malarias will be continued until an adequate sample of each species has been investigated. The study will be extended, largely through cooperation with other malaria research agencies, to other Asian and Malaysian countries. Samples from ecologically distinct environments will be expanded. A monographic account of the malarias of the Asian primates will be prepared.

Part B included - Yes

Serial No. NIAID-135-B

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Lambrecht, F.L., Dunn, F.L. and Eyles, D.E. Isolation of *Plasmodium knowlesi* from Philippine macaques. Nature (in press)

LABORATORY OF IMMUNOLOGY

Summary 1

140 - Studies in the Transplantation of Immunologically Competent Tissues 5
141 - Studies on Antibody Production and Mechanisms of Hypersensitivity 8
142 - The Immunology of C_3h Mouse High Line Sarcoma 11
145 - Studies of Auto-Antibodies in Human Disease States and in Experimental Animals 13
145-A - Study of the Antigens in Serum by Immunochemical Techniques 18
147 - Immunochemical and Immunogenetic Studies of the Protein Allotypes in Serum 20
147-A - Immunochemical Studies on Human Serum Antigens and Antibodies 26
148 - Basic Studies on the Cellular Localization of Antigens, Antibodies and other Substances by the Fluorescent Antibody Technique and Fluorescence Microscopy 29
150 - The Relationship of Delayed and Immediate Hypersensitivity to Allergic Thyroiditis in Inbred Guinea Pigs 34
151 - The Purification and Characterization of a Proteolytic Enzyme of Human Spleen 37
152 - The Chemistry of Antibodies 39
153 - Basic Studies on the Chemical, Physical and Skin Reactive Properties of Extracts of House Dust 43
154 - Immunocytochemical Studies of the Reactivity of Human Serum Proteins with Human Tissues in Normal and Disease States 46
155 - Cellular Production of Gamma Globulin; Studies of Rabbit Allotypes Using the Fluorescent Antibody Technique 48
156 - The Study of Delayed Hypersensitivity _In Vitro_ 50

PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
Calendar Year 1961

Over a period of the last two years the Laboratory has lost three of its senior scientific staff. This loss leaves certain gaps in our program which should be filled in the near future as soon as adequate space becomes available. Due to the fact that the Basic Sections of the Laboratory did not occupy the renovated space in building #5 but are remaining in building #10, the necessary laboratory space and facilities are not available at the present time for the addition of permanent professional personnel. However, it is my conviction that as soon as the assigned space is ready for occupancy we should think in terms of adding scientists to several of the Sections. The majority of these should be in the younger Ph.D. category functioning as "lieutenants" for the senior scientists which are presently Section Heads. There is no problem recruiting investigators in the younger M.D. category. This was brought to our attention again this year in a most forceful manner in the Research Associate Program. Of the candidates applying for positions in NIAID it is estimated that approximately 85 - 90 percent selected the Laboratory of Immunology as their first or second choice. Many of these were well along in their training having one or more years of residency. Furthermore, we have almost weekly requests from young physicians desiring training in our Laboratory for a period of two years. Their desire is influenced in part by the fact that service here at the NIH satisfies their military requirements. However, the majority of them foresee the importance of immunology and the application of the newer immunological techniques to the various fields of medicine.

During the past year the majority of the scientists of the Laboratory of Immunology have taken on considerable responsibility in the training of Clinical Associates from the Laboratory of Clinical Investigation. This has been a healthy move but at times it has taxed our senior personnel. The addition of several permanent personnel would aid considerably in keeping our regular program running smoothly toward our goals. At the present writing, six Clinical Associates from LCI are carrying on studies in our Laboratory under the supervision of our senior scientific staff. Besides these six physicians, we have assigned to us an M.D. filling a staff position, a Research Associate and a Research Fellow which require considerable attention and training. These three are here on a two-year basis until July of 1963. Another temporary member of our laboratory, a Visiting Scientist, will be here until September of 1962. At the present time the Laboratory of Immunology has only six permanent staff members, one of which has been abroad for training during the past year. Considering that five of our staff members have been providing training for nine or more young physicians, it seems apparent that an imbalance has been reached. It is essential that we keep the proportion of permanent and temporary scientists in proper balance. Furthermore, it is my considered opinion that our Laboratory can be even more produc-

tive if we have more permanent staff members and fewer scientists here on a temporary basis. The backbone of our continuing program will be the scientists who are here on a long term basis.

The weekly immunology seminars initiated in 1960 have continued to serve a very worthwhile function during 1961. The various groups and individuals, concerned in immunological studies throughout the various Institutes, have made it possible for us to have an outstanding program with speakers from this country and abroad. The seminars consistently have been well attended. This year a seminar was dedicated to our first Chief, Dr. Jules Freund, and at this function more than four hundred people were in attendance. This serves as an indication of the intense interest in immunology which is being shown by the scientists here at the National Institutes of Health.

RESEARCH ACCOMPLISHMENTS

PASSIVE TRANSFER OF ALLERGIC ENCEPHALOMYELITIS

The passive transfer of allergic encephalomyelitis was accomplished when lymph node cells from sensitized Strain 13 inbred guinea pigs were transferred to normal Strain 13 recipients. Furthermore, this experimental autoimmune disease may be transferred from sensitized adult Strain 13 guinea pigs to newborn Strain 13 animals. Strain 2 inbred guinea pigs are relatively resistant to allergic encephalomyelitis and the disease is not transferred to the F1 generation animals resulting from a Strain 2 - 13 cross. The F1 animals evidently inherit the Strain 2 resistance. Passive transfer with viable lymphoid cells represents an important tool in the study of immune phenomena. In histocompatible animals, the variable of transplantation immunity is eliminated, the transferred cells thrive, and the cells go on to immune maturity in the recipient. With the strategic use of Strain 2, Strain 13 and their F1 hybrids, crucial problems concerning the mechanism of hypersensitivity may be attacked for the first time.

ANTIBODY PRODUCTION AND GAMMA GLOBULIN IN HUMAN MALARIA

For the first time, the course of antibody production has been followed in human malaria. Although there has been no practical serological test for the detection of antibody produced in human malaria infections, it has been possible not only to detect but to titrate antibody by the fluorescent antibody technique and to correlate this with the appearance of the parasites in the peripheral circulation. In 4 sporozoite-induced Plasmodium vivax infections in human volunteers, antibody was detectable several days after the parasites appeared. In a typical case, the antibody rapidly rose to a serum dilution level of 1:2,560, was maintained at this level for about 40 days after which it gradually fell and remained at a low level for the period of observation (121 days). In 5 patients infected with the B strain of

P. cynomolgi essentially the same results were obtained and certain of the patients still had detectable antibody about 1 year after infection. The patients showed increased gamma globulin levels above the normal limits. The maximum gamma globulin rise tended to occur at the same time as the maximum antibody titer with a median difference of 3 days. The ability to follow antibody production and increases in gamma globulin levels in controlled malaria infections provides some very basic information necessary in the program connected with the worldwide eradication of malaria.

PROTEOLYTIC ENZYME OF HUMAN SPLEEN PURIFIED — A method has been established for the isolation and purification of a proteolytic enzyme from human spleen which cuts human albumin into large precipitating fragments. By column chromatography it has been shown to be a single type of substance. Characterization of the enzyme as to electrophoretic mobility, pH activity curve and catheptic activity has been determined. Four large fragments of human albumin resulting from the degradation of the native protein by the human enzyme have been detected by immunoelectrophoresis. This study combines several fundamental problems bearing on the nature of antigenicity and the possible roles of enzymes in the degradation of antigens as a primary step in antibody formation.

LUPUS SERUM REACTS WITH MAMMALIAN CHROMOSOMES — Fluorescent antibody studies have demonstrated that when sera from certain patients having lupus erythematosus were applied to mammalian chromosome preparations, these sera reacted as was evidenced by their fluorescence. The sera from the lupus patients had been shown to have anti-nuclear factors. In the case of human chromosomal preparations all 46 chromosomes reacted and became fluorescent. When sera from three patients with Sjögrens syndrome were utilized, the chromosomes did not become fluorescent even though the sera had anti-nuclear factors. It may be possible to find sera or fractions of sera which are more specific in their chromosomal reactions. The anti-nuclear factors found in the sera of patients with the so-called autoimmune diseases are of importance for a better understanding of the mechanisms involved.

GENETIC CONTROL OF GAMMA GLOBULIN ALLOTYPES — In studies on the genetic control of serum allotypes in rabbits, it was previously shown that RGG-I and RGG-II allotypic determinants are under the genetic control of an allelic pair of autosomal genes. Further studies have revealed three other gamma globulin allotypic determinants which are under the genetic control of a three allelic system at a second gene locus. Furthermore, two allotypic determinants were either both present or absent from all sera tested indicating genetic control by a single allele or two closely linked alleles. In addition a gamma globulin at a third locus has been found. The distribution of allotypes varied considerably among several rabbit colonies. The selective breeding of rabbits is in progress to establish genetically defined

lines of rabbits based on the known gamma globulin allotypes. To know the genetic mechanism for the control of allotypes is essential for the usefulness of these allotypes in studies of basic immunology and hypersensitivity.

ALLERGIC THYROIDITIS Delayed hypersensitivity to thyroid extract has been successfully transferred within Strain 13 inbred guinea pigs. Intact lymph node cells from animals actively immunized to thyroid extract were injected into normal recipients. Successful transfer was accomplished only if the lymph node cells were taken early after immunization of the donors. Cells taken 5 days after sensitization successfully transferred skin test hypersensitivity. Lesions of the thyroid were produced by the transfer of lymph node cells in about 30 percent of the recipients. The animal models selected for the study of the autoimmune diseases should aid in the elucidation of the role of circulating antibodies and delayed hypersensitivity in the production of tissue lesions. As more is known about the pathogenesis of these diseases the more intelligent we can be in our approach to the therapy of the autoimmune diseases.

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 140
1. Laboratory of Immunology
2. Natural and Acquired Resistance
3. Bethesda, Maryland

Part A:

Project Title: Studies in the Transplantation of Immunologically Competent Tissues

Principal Investigator: Dr. Sanford H. Stone

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Project Subtitle I: Isologous and Homologous Lymphoid Cell Transplants

Objectives:

To study the passive (adoptive) transfer of tuberculin sensitivity, antibody producing activity and autoimmune disease inducing capacity by sensitized lymph node cells between histocompatible and histoincompatible guinea pigs.

Methods Employed:

Inbred guinea pigs are sensitized with egg albumin or guinea pig spinal cord in Freund's complete adjuvant (containing mycobacteria). Lymphoid cell transfers are made by intraperitoneal route into inbred or random-bred guinea pigs or into the F1 generation of a Strain 2-Strain 13 cross. Cell recipients are tested for skin-reactivity, circulating antibody or observed for signs of autoimmune disease. Tissues are taken from spinal cord or brain of guinea pig recipients with allergic encephalomyelitis for histological examination.

Major Findings:

1. Transfers of tuberculin hypersensitivity and antibody synthesizing capacity appears to be effective between Strain 2 or Strain 13 donors and the F1 guinea pigs conceived of their crossing as well as within the strains.

2. A typical experimental autoimmune disease, allergic encephalomyelitis is transferred by lymph node cells of sensitized Strain 13 guinea pigs to normal Strain 13 recipients, but not to F1 animals of the 2-13 cross, emphasizing the differences in reactivity between the inbred strains of guinea pig (Strain 2 is relatively resistant to allergic encephalomyelitis, and the 2-13 cross evidently inherits this resistance).

3. Allergic encephalomyelitis may be transferred from sensitized adult Strain 13 animals to newborn Strain 13's. The "resistance" of newborn guinea pigs to manifestations of delayed type hypersensitivity does not hold in this case, implying that reactivity of the skin may explain the general experience in studies of hypersensitivity in immature animals.

Significance to the Program of the Institute:

Passive transfer with viable lymphoid cells represents an important tool in the study of immune phenomena. In histocompatible animals, the variable of transplantation immunity is eliminated, the transferred cells thrive, and go on to immune maturity in the recipient. With strategic use of the Strain 2, Strain 13 and their F1 hybrid, crucial problems concerning the mechanism of hypersensitivity may be attacked for the first time.

Proposed Course of Project:

Further studies will be undertaken using the F1 generation guinea pigs of the Strain 2-Strain 13 cross in problems concerned with nature and fate of the cell lines responsible for the activity in the recipient.

Part B included: Yes X No

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications:

Bauer, J. A., Jr. and Stone, S. H.: Isologous and Homologous Lymphoid Transplants I. The Transfer of Tuberculin Hypersensitivity in Inbred Guinea Pigs. J. Immunol. 85:177, 1961

Stone, Sanford H.: Transfer of Allergic Encephalomyelitis by Lymph Node Cells in Inbred Guinea Pigs. Science 134:619, 1961

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 141
1. Laboratory of Immunology
2. Natural and Acquired Resistance
3. Bethesda, Maryland

Part A:

Project Title: Studies on Antibody Production and Mechanisms of Hypersensitivity

Principal Investigator: Dr. Sanford H. Stone

Other Investigators: None

Cooperating Unit: None

Man Years:

Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Project Subtitle I: Antibody Levels and Degree of Delayed Hypersensitivity In Systemic Allergic Phenomena and Autoimmune Diseases

Objectives:

To study the mechanisms of "immediate" and "delayed" hypersensitivity

Methods Employed:

Inbred histocompatible guinea pigs permit experimentation on genetic factors involved in hypersensitivity. The experimental models: random-bred or inbred guinea pigs injected with antigen in complete (for "delayed" hypersensitivity studies) or incomplete (for "immediate" hypersensitivity studies) Freund's adjuvants, and challenged by the subcutaneous route with homologous antigen. Methods of quantitation of the manifestations of "immediate" (survival time in protracted anaphylaxis) and "delayed" (amount of mycobacterium necessary to sensitize) hypersensitivity become feasible. The local massive hemorrhagic reaction described in previous reports is most versatile for manifestation of "delayed" hypersensitivity; the allergic encephalomyelitis syndrome for manifestation of an autoimmune disease.

Major Findings:

1. Studies were continued on sex and strain differences in reactivity in random-bred and inbred guinea pigs. Contrary to the results obtained with the delayed hemorrhagic reaction, Hartley guinea pigs were only slightly more susceptible to tuberculin sensitization than the inbred

strains 2 and 13. The higher susceptibility of females seen in the hemorrhagic reaction was not found for tuberculin reactivity. In the active inducing of allergic encephalomyelitis, Hartley and Strain 13 guinea pigs were considerably more susceptible than Strain 2 guinea pigs.

2. Some progress in the elucidation of the mechanism of the delayed hemorrhagic reaction can be reported: guinea pigs sensitized with egg albumin in complete or incomplete Freunds adjuvants both undergo a formidable decrease in circulating platelets during the protracted shock syndrome which follows a subcutaneous injection of a large amount of antigen; however, only in those sensitized using the complete adjuvant (containing mycobacteria) does the hemorrhage appear at the site of subcutaneous deposition of antigen. Evidently, another factor (associated with delayed sensitivity) triggers the massive hemorrhagic response.

Significance to the Program of the Institute:

Some clarification of the mechanism of anaphylactic shock may be possible, and some unification of current theories on mechanism may be achieved. Some comparison of the roles of delayed and immediate hypersensitivity components in systemic allergic phenomena and autoimmune diseases may be feasible from these studies.

Proposed Course of Project:

The mechanisms of protracted anaphylaxis and the local hemorrhagic reaction will be investigated using random-bred and inbred guinea pigs.

Part B included: Yes X No

Part B: Honors, Awards, and Publications

Publications:

Stone, Sanford H.: Acute and Protracted Anaphylactic Shock After Subcutaneous Challenge in Random-Bred and Inbred Guinea Pigs. Internat. Arch. Allergy Applied Immunol. 19:227, 1961

Serial No. NIAID - 142

Proposed Course of Project:

Discontinued because of negative results.

Part B included: Yes No X

[illegible] Serial No. [illegible]
[illegible] PHS-NIH 1. Laboratory of [illegible]
Individual Project Report 2. [illegible]
Calendar Year [illegible] 3. Bethesda, Maryland

Part A

Project Title: [illegible]

Principal Investigator: Dr. [illegible]

Other Investigators: Dr. [illegible]
Dr. [illegible]
Dr. [illegible]

Cooperating Units:

Dr. [illegible]

Dr. [illegible]

Man Years

Total: [illegible]
Professional: [illegible]
Other: [illegible]

Project Description

Objectives

1. [illegible]

2. [illegible]

3. [illegible]

4. [illegible]

Methods Employed

[illegible]

and characterize the serum factors which react with tissue antigens.

To produce auto-antibodies in animals experimentally, rabbits, guinea pigs and rats are immunized with tissue extracts incorporated in Freund's adjuvant.

Patient Material:

Patients with lupus erythematosus and idiopathic nephrotic syndrome and other diseases where auto-immune phenomena are found or suspected are admitted to the Clinical Center for these studies. Kidney biopsies are performed and the clinical response to steroid administration or to the administration of the purine anti-metabolites 6-Mercaptopurine or 6-Thioguanine is studied: lipid metabolism studies on patients with the nephrotic syndrome have been performed in conjunction with Dr. James Baxter, NHI.

Major Findings:

1. Detection and characterization of factors in human sera that react with human tissue antigens.

   Drs. Krooth, Tobie, Tjio and I have shown that certain of the human sera which react with cell nuclei also react with the chromosomes of the cell nucleus; other sera which react with cell nuclei do not react with the chromosomes. DEAE cellulose chromatography of one such serum showed that the anti-chromosome reactivity was contained in the 7S γ-globulins and in a second fraction which also contained the 18-19S (β-2M) macroglobulins.

2. Characterization of tissue antigens.

   Dr. Jose Uriel has recently joined our laboratory as a visiting scientist. Since these studies have been initiated so recently no major findings can be reported.

3. Production of auto-antibodies in experimental animals.

   Studies are now in progress to determine whether thyroglobulin is the antigen responsible for production of auto-allergic thyroiditis. Guinea pigs immunized with crude thyroglobulin do show skin test hypersensitivity to purified thyroglobulin; guinea pigs are now being immunized with purified thyroglobulin to determine whether auto-allergic thyroiditis is produced.

   Attempts were made to reproduce the anti-nucleus factors found in the serum of patients with lupus erythematosus and other diseases. In confirmation of the results we obtained in rabbits, guinea pigs immunized with autologous (guinea-pig) nucleoprotein extracts did not develop detectable auto-antibodies. When immunized with foreign

nucleoprotein extracts (calf thymus or human liver) incorporated in Freund's adjuvant, guinea pigs developed antibodies not only to the foreign extracts but made antibodies which cross-react with guinea pig extracts as well. In two species, therefore, rabbit and guinea pig, auto-antibodies were not produced by injection of homologous tissue antigens, but were produced after immunization with foreign tissue extracts known to contain antigenic groupings in common with the recipient's tissue antigens. In the rabbits, the auto-antibodies are not directed against nucleoproteins (agar-gel precipitin lines characterized by Dr. Uriel), although at least certain of the sera do react with rabbit cell nuclei.

4. Production of auto-immune tissue lesions in experimental animals.

a) These studies were begun by Drs. McMaster and Lerner and continued in collaboration with Dr. Stone to determine whether tissue lesions in auto-immune thyroiditis were due to the delayed type of hypersensitivity. Inbred guinea pigs of Strain 13 were actively immunized with Strain 13 guinea pig thyroid extract, after which intact lymph node cells from the actively immunized donors were injected into normal Strain 13 recipients.

The delayed hypersensitivity skin test to thyroid extract can be successfully transferred only if the lymph node cells are taken early after immunization of the donors; cells taken 5 days after sensitization successfully transferred skin test hypersensitivity to thyroid extract, while cells taken 9 and 12 days after immunization did not transfer skin test hypersensitivity. Lesions have been produced successfully by transfer of the lymph node cells in about 30% of recipients. However, PPD hypersensitivity was successfully transferred in most of the recipients.

b) Studies of the mechanism by which cells may be transferring the auto-immune tissue lesions were continued with Dr. John Turk of Dr. John Humphrey's Division of Immunology at Mill Hill, England, during a one month visit in November. During this period a cell-free extract of lymph nodes and suspensions of cell microsomes were used in transfer experiments in our laboratories, in collaboration with Drs. Stone and Lerner. Attempts were made to transfer the skin-test hypersensitivity and produce lesions of auto-allergic thyroiditis and of allergic encephalomyelitis. Preliminary results indicate that the cell sap and microsomes transfer an Arthus-like reaction to thyroid extract and PPD rather than the delayed type of hypersensitivity.

c) Dr. Sheldon Wolff has immunized Strain 13 guinea pigs with adrenal glands (obtained from other strains of guinea pigs) incorporated in Freund's adjuvant. Immunized animals have developed positive skin tests indicating delayed type hypersensitivity to Strain 13 adrenal gland extracts.

Significance to the Program of the Institute:

Substantial progress has been made in developing a program to study the auto-immune phenomena in human disease states and experimental animals. The success of this program depends upon a coordinated approach from the clinical to the most basic immunological level. The program, therefore, has been developed in collaboration with Dr. Vernon Knight in the Laboratory of Clinical Investigation and with several of the members of the Laboratory of Immunology.

The importance of auto-antibodies in the pathogenesis of a number of human diseases deserves critical study. The animal models of auto-immune diseases have been selected to help elucidate the role of circulating antibodies and of delayed hypersensitivity in the production of the tissue lesions. As more is known about the pathogenesis of the auto-immune tissue lesions, a more intelligent approach to therapy of these conditions can be developed. Meanwhile studies of the effects of administration of steroid hormones and purine anti-metabolites (6-Mercaptopurine and 6-Thioguanine) on the course of diseases in which auto-immune phenomena are detectable will continue to provide valuable information about therapy of these diseases and possibly also help indicate whether the auto-immune phenomena play a role in pathogenesis of the diseases.

Proposed Course of Project:

Attempts to detect auto-antibodies in different disease states will continue. The auto-antibodies detected will be characterized by immunoelectrophoresis and cellulose column chromatography. Animal studies on the production of and characterization of auto-antibodies and production of tissue damage will continue, with emphasis on the antibodies to components of cell nuclei, to thyroid tissue, and to adrenals and kidneys. In patients and in experimental animals where tissue damage is associated with auto-antibodies, attempts will be made to determine whether tissue damage is caused by hypersensitivity of the delayed type or due to circulating antibodies. Cell and serum transfer experiments can help decide this question.

In addition, experiments are planned to determine the possible significance as a general model for the formation of auto-antibodies of the interesting observations that guinea pigs and rabbits will make auto-antibodies to certain native antigens when these antigens are present in tissue extracts from another species, immunization with autologous tissue extracts.

Part B included: Yes X No

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications:

Allen, James C., Baxter, James H. and Goodman, Howard C. Effects of Dextran, Polyvinylpyrrolidone and Gamma-Globulin on the Hyperlipidemia of Experimental Nephrosis. J. Clin. Inv. 40:449, 1961.

Baxter, James H., Goodman, Howard C. and Allen, James C. Effects of Infusions of Serum Albumin on Serum Lipids and Lipoproteins in Nephrosis. J. Clin. Inv. 40:490, 1961.

De Vaux St. Cyr, Ch., Cleve, H., Goodman, H. and Grabar, P. Etude Immunoelectrophoretique des Proteines des Urines Normales et Pathologiques Fractionnees sur Colonne de Cellulose DEAE. Protides of the Biological Fluids. Proceedings of the 8th Colloquium. 65, 1961.

Goodman, Howard C. Immunoelectrophoretic Studies of the Gamma Globulins in Normal Urine and in Urine from Patients with the Nephrotic Syndrome. Proceedings of the Twelfth Annual Conference on The Nephrotic Syndrome. 245, 1961.

Goodman, Howard C., De Vaux St. Cyr, Christiane, Cleve, Hartwig and Grabar, Pierre. Immunoelectrophoretic Studies of Normal Human Serum Fractionated by Anion Exchange Cellulose Column Chromatography. Protides of the Biological Fluids, Proceedings of the 8th Colloquium. 135, 1961.

Krooth, Robert S., Tobie, John E., Tjio, J. H., Goodman, Howard C. Reaction of Human Sera with Mammalian Chromosomes Shown by Fluorescent Antibody Technique. 134:284, 1961.

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 145-A
1. Laboratory of Immunology
2. Clinical Immunology
3. Bethesda, Maryland

Part A:

Project Title: Study of the Antigens in Serum by Immunochemical Techniques

Principal Investigator: Dr. Howard C. Goodman

Other Investigators: None

Cooperating Unit:

Dr. Donald Frederickson, LCPM, NHI

Man Years:

Total: 0.1
Professional: 0.1
Other: None

Project Description:

Objectives:

To apply immunochemical methods to aid in the study of the serum protein in normal human serum and the serum protein abnormalities in disease states.

Methods Employed:

Gel-diffusion techniques and immunoelectrophoresis.

Major Findings:

We have begun to use immunoelectrophoresis for characterization of serum proteins in diseases other than those caused by immune reactions. With Dr. Frederickson, (NHI), we demonstrated for the first time the marked (50-100 fold) decrease of the high density (alpha) lipoproteins in the serum of two patients with a previously undescribed lipid deposition disease which Dr. Frederickson has named "Tangier Disease" because the first two patients were found in Tangier Island, Chesapeake Bay. We also demonstrated the absence of the low-density beta lipoproteins in the sera of two patients with the rare syndrome of "a-betalipoproteinemia".

Significance to the Program of the Institute:

Immunoelectrophoresis is a powerful tool for the study of qualitative

and semiquantitative changes in serum protein constituents in disease states. These studies are an example of the growing importance of the application of immunochemical techniques to problems outside the field of those diseases associated with hypersensitivity states.

Proposed Course of Project:

Continuation of characterization of serum lipoprotein abnormalities with Dr. Frederickson.

Part B included: Yes No X

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 147
1. Laboratory of Immunology
2. Immunochemistry Section
3. Bethesda, Maryland

Part A:

Project Title: Immunochemical and Immunogenetic Studies of the Protein Allotypes in Serum

Principal Investigator: Dr. Sheldon Dray

Other Investigators: Miss Glendowlyn Young

Cooperating Units:

Project Subtitle I - Dr. Charles W. McPherson, Animal Production Section, Serial No. (not reported by LAB)

Project Subtitle II - Dr. Jacques Oudin, Pasteur Institute, France; Dr. S. Dubiski, Institute of Hematology, Warsaw, Poland; Dr. Edwin Lennox, New York University, New York, N. Y.; and Dr. A. Kelus, Department of Pathology, Cambridge University, Cambridge, England.

Project Subtitle V - Dr. T. N. Harris, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

Project Subtitle VI - Dr. Fred Karush, University of Pennsylvania, Philadelphia, Pennsylvania

Man Years:

Total: 3.0
Professional: 1.5
Other: 1.5

Project Description:

Project Subtitle I: Genetic Control of Serum Allotypes

Objectives:

To establish the genetic basis for the presence of serum allotypes and to develop lines of animals (rabbits) with genetically defined allotypes.

Methods Employed:

1. Qualitative tests using primarily agar gel methods for the identification of allotypes in families of rabbits and in various rabbit populations.

2. Selective breeding of rabbits to test genetic hypotheses and establish genetically defined lines.

Major Findings:

In addition to the RGG-I and RGG-II allotypic determinants previously described which are under the genetic control of an allelic pair of autosomal genes, family studies revealed three other gamma globulin allotypic determinants which are under the genetic control of a three allelic system at a second gene locus. Furthermore, two allotypic determinants were either both present or absent from all sera tested indicating genetic control by a single allele or two closely linked alleles. The distribution of allotypes varied considerably among several rabbit colonies. In addition, a gamma globulin at a 3rd locus has been found.

Selective breeding of rabbits has been carried out to establish genetically defined lines of rabbits based on the known gamma globulin allotypes. Among the NIH New Zealand Whites, a homozygous II L line has already been established. A homozygous II K line should become established in about six months to one year. The homozygous IJ, IK, and IL lines will take somewhat longer to develop, perhaps one or two years, since these genes segregate only in the small NIH Flemish Giant Colony.

Significance to the Program of the Institute:

To know the genetic mechanism for the control of allotypes is essential for the usefulness of these allotypes in studies of basic immunology and hypersensitivity. The molecules having antigenic sites under the genetic control postulated are soluble proteins, namely, gamma globulins. Some of these molecules may also react as antibodies. As antigens, precipitable by antibodies, they should be subject to quantitative estimation. Moreover, these molecules may be expected to pass through maternal-fetal barriers. Thus, this immunochemical genetic system may be uniquely suited for studies of some basic problems in immunology, hypersensitivity, embryology, and protein chemistry.

Proposed Course of Project:

1. To establish genetically defined lines of rabbit gamma globulin types so that they may be available for other studies.

2. To study the genetic control of new gamma globulin allotypes and other allotypes as they are discovered.

Project Subtitle II: Nomenclature of Rabbit Serum Allotypes

Objectives:

To establish a uniform nomenclature for the serum allotypes in rabbits.

Methods Employed:

1. Comparison of antigens and antibodies developed in different laboratories.

2. Preparation of standard antigens and antisera.

3. Conference of principal investigators to establish a uniform nomenclature.

Major Findings:

The antisera developed by Dr.'s Oudin, Dray, and Dubiski were compared and their identification established. Requirements for standard antigens and antisera were set up and a uniform nomenclature proposed. Procedures for naming new allotypes as they are discovered was agreed upon. The results of this conference will be submitted for publication soon.

Significance to the Program of the Institute:

A uniform nomenclature is essential to minimize confusion among research workers. The problem is analagous to that of naming the blood groups.

Proposed Course of Project:

To continue cooperation among investigators so that reference antigens and antisera may be available and that a uniform nomenclature may be used.

Project Subtitle III: Chemical Studies of Serum Allotypes

Objectives:

1. To identify the antigens in serum which induce allotypes.

2. To study the chemical nature of these allotypes.

Methods Employed:

1. Immunochemical analysis by diffusion in agar gel; simple diffusion in plates, and immunoelectrophoresis.

2. Passive cutaneous anaphylaxis, tanned-cell hemagglutination and other immunological tests for antibody.

3. Cellulose ion-exchange chromatography and other protein fractionation and purification methods.

4. Enzyme (papain) digestion of allotypes to produce smaller fragments for study.

Major Findings:

Investigations have shown that components of sera from individual rabbits are antigenic in certain other rabbits and that isoprecipitins can be produced which react with serum antigens having electrophoretic mobilities corresponding to alpha-, beta-, and gamma globulins.

Significance to the Program of the Institute:

1. Differences in serum proteins between individuals of the same species may be of genetic and clinical significance similar to that of the blood groups. The serum allotypes may be implicated in some of the unexplained transfusion reactions in man.

2. Serum allotypes may be useful in the study of "immune tolerance" and perhaps serve as a somewhat analagous experimental model for the study of supposed tolerance to Rh antigen.

3. Serum protein allotypes may be particularly suitable for study of some basic problems in immunology concerning the nature of antigen and antibody sites, antigenicity of antibodies, chemical structure of gamma globulin, the antigen-antibody reaction, etc.

Proposed Course of Project:

1. Chemical characterization of the gamma globulin allotypes in rabbits, and the development of methods of assay for each of the allotypes.

2. Studies on the chemical structure of gamma globulin by papain digestion studies and quantitative assay of antigenic sites.

3. Search for additional gamma globulin allotypes and additional alpha and beta globulin allotypes.

4. Specificity of rabbit allotypes and antigenicity in other species.

Project Subtitle IV: Synthesis of Gamma Globulins in The Fetus and Newborn From Normal and Immunized Mothers

Objectives:

1. To determine when and where gamma globulins are first produced by the fetal or neonatal rabbit and the rate at which the gamma globulin synthesized by the offspring appears in the serum.

2. To study the effect of antibody in the mother on the production of gamma globulin in the offspring.

Methods Employed:

1. Selective breeding of rabbits on the basis of the known genetics of gamma globulin antigens.

2. Immunochemical methods to assay quantity of gamma globulin allotype appearing in the serum.

3. Fluorescent antibody to localize time and site of appearance in tissues.

Major Findings:

Preliminary findings have shown that neonatal rabbits at two weeks of age are producing gamma globulin allotypes based on their own genetic constitution. The quantity of these gamma globulin allotypes increases rapidly, reaches a peak and levels off at about 12 - 16 weeks.

Significance to the Program of the Institute:

1. The allotype-isoantibody system in rabbits provides the best means for study of gamma globulin formation in the newborn animals since the gamma globulin produced by the offspring can be clearly distinguished from maternal gamma globulins which pass the placental barriers.

2. May elucidate basic mechanisms involved in gamma globulin and antibody formation.

Proposed Course of Project:

The possibility for the study of the rate of gamma globulin synthesis per cell.

Project Subtitle V: Use of allotypes as Cell Markers in Cell Transfer Studies

Objectives:

To determine whether donor white cells from an immunized rabbit produce antibody in an irradiated recipient.

Methods Employed:

1. Use of gamma globulin typed rabbits as donors and recipients.

2. Fluorescent antibody to identify the source of gamma globulin antibody found in the recipient.

Major Findings: None

Significance to the Program of the Institute:

The allotype-isoantibody system provides the means to definitively determine the above objectives, and would demonstrate the applicability of this system to other problems in immunology involving cell transfer.

Proposed Course of Project:

To apply this technique to problems involving cell transfer such as in producing chimera.

Project Subtitle VI: Antibody Heterogeneity Relationship of Allotypes to Antibody Production

Objectives:

To determine whether antibody prepared against a simple antigenetic determinant in a rabbit with several allotypes has all these allotypes or whether selection of allotypes has occurred.

Methods Employed:

1. Immunization of rabbit heterozygotes with haptens conjugated to proteins.
2. Preparation of pure antibody to haptens.
3. Analysis of pure antibody for allotypes.

Major Findings:

Preliminary study of antibody to albumin showed no selection for allotypes.

Significance to the Program of the Institute:

The results of this experiment are of particular interest concerning the question of heterogeneity of antibodies, and the clonal theory of antibody production.

Proposed Course of Project:

Studies are in progress and will be continued.

Part B included: Yes No X

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 147-A
1. Laboratory of Immunology
2. Immunochemistry Section
3. Bethesda, Maryland

Part A:

Project Title: Immunochemical Studies on Human Serum Antigens and Antibodies

Principal Investigator: Dr. Sheldon Dray

Other Investigators: Dr. Edward Lichter, Post-Doctoral Fellow

Cooperating Unit:

Project Subtitle I - Dr. John Fahey, Metabolism Branch, Serial No. NCI -

Project Subtitle II - Dr. Baruch S. Blumberg and Dr. Joseph C. Robinson, EBB, Serial No. NIAMD -

Man Years:

Total: 2.5
Professional: 1.0
Other: 1.5

Project Subtitle I: Studies With Primate Antisera

Objectives:

1. To develop antisera specific for antigenic determinants on serum proteins.

2. To look for allotypes in human sera.

Methods Employed:

1. Agar gel immunization methods.

2. Purification and fractionation of normal and pathologic serum proteins.

3. Enzyme digestion of serum proteins to obtain fragments.

4. Immunization of primates (Rhesus monkeys and chimpanzees).

Major Findings:

1. The immunochemical relationship between the three normal 7S γ-globulin antigens and two myeloma γ-globulins were investigated and it was found that one of the myeloma γ-globulins corresponded to one of the

One of the changing antisera has precipitating antibodies to two γ-globulins.

Significance to the Program of the Institute:

Individual differences in man of the antigenic specificity of serum proteins are of particular interest from the point of view of possible blood transfusion reactions and from the point of view of genetic and anthropological studies.

Proposed Course of Project:

These studies are in progress and will be continued.

Part B included: Yes No X

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 148
1. Laboratory of Immunology
2. Applied Immunology
3. Bethesda, Maryland

Part A:

Project Title: Basic Studies on the Cellular Localization of Antigens, Antibodies and other Substances by the Fluorescent Antibody Technique and Fluorescence Microscopy.

Principal Investigator: Dr. John E. Tobie

Other Investigators: None

Cooperating Units:

Project Subtitle I - Dr. Sanford F. Kuvin, LCI, Serial No. NIAID - 20 (c), Dr. G. Robert Coatney, LPC, Serial No. NIAID - 130

Project Subtitle II - Dr. Robert S. Krooth, LVB, Serial No. NIAID - (not reported by LBV)

Project Subtitle III - Mrs. Eleanor J. Tobie, LPD, Serial No. NIAID - 123-D

Man Years:

Total: 3.0
Professional: 1.0
Other: 2.0

Project Description:

Project Subtitle I: Fluorescent Antibody Studies in Malaria

Objectives:

To specifically stain human malaria parasites by the fluorescent antibody technique.

To devise a fluorescent antibody method for the detection of malarial antibody produced in human volunteers infected with the human and monkey malarias.

To correlate antibody production with gamma globulin levels.

To study the cross-reactions between the various species of malaria found in man.

Methods Employed:

Globulin fractions of sera from humans infected with *Plasmodium vivax* and *P. cynomolgi bastianellii* were conjugated with fluorescein isothiocyanate and the conjugates applied to blood films containing the malaria parasites. The films were then examined at fluorescence microscopy.

Antibody production in human volunteers infected with malarial parasites was followed by the indirect fluorescent antibody technique. Sera from infected patients were allowed to react with malarial organisms in blood films and then fluorescent labeled horse anti-human globulin applied.

Gamma globulin levels in infected patients were determined by electrophoretic methods and the levels correlated with the rise in malarial antibody as determined by fluorescent methods.

Cross-reactions between the various species of malaria will be studied by the indirect fluorescent antibody technique utilizing the sera of patients and titering these sera against the different malarial organisms.

Major Findings:

Plasmodium vivax and *P. cynomolgi bastianellii* have been stained successfully. Although there has been no practical serological test for the detection of antibody produced in human malarial infections, it has been possible to detect and to titer the antibody by the fluorescent antibody technique and to correlate this with the appearance of the parasites in the peripheral circulation. In two sporozoite-induced *P. vivax* infections, antibody was detectable several days after the parasites appeared. In one patient the antibody rapidly rose to a serum dilution level of 1:320 and in the other to a level of 1:2,560. These levels were maintained for about 60 days after which they gradually fell and were then maintained at low levels to 121 days, 1:20 and 1:60 respectively. In 5 patients infected with *P. cynomolgi bastianellii* essentially the same results were obtained and certain of the patients still had detectable antibody 1 year after infection. Preliminary observations on the above patients suggest that there is a correlation between the rise in gamma globulin levels and malaria antibody levels. On a limited number of cases the cross reactions between *P. vivax* and *P. cynomolgi bastianellii* have been studied. It appears that these two species which are so similar morphologically are quite similar serologically.

Significance to the Program of the Institute:

The ability to specifically stain malaria parasites and to accurately follow antibody production in controlled infections provides some very basic information necessary in the program connected with the worldwide eradication of malaria. A study of the serological relationships between malaria plasmodia of human and animal origin are of great importance with the possibility that monkeys may serve as reservoirs of human infection.

Proposed Course of Project:

To continue studies on antibody production and gamma globulin levels in patients infected with the various species of malaria as well as to study the serological relationships of the malaria plasmodia.

Project Subtitle II: Reaction of Human Sera with Mammalian Chromosomes

Objectives:

To determine if certain human sera having anti-nuclear factors will react immunologically with not only the nucleus but also with a component of the nucleus - the chromosomes.

Methods Employed:

Human sera containing anti-nuclear factors were applied to chromosomes prepared from peripheral blood cultures and tissue cultures and allowed to react. Fluorescent labeled horse anti-human globulin was then applied and the chromosomes examined at fluorescence microscopy.

Major Findings:

Of six sera from patients with lupus erythematosus, five reacted with mammalian chromosomes. In the case of human chromosomes it was demonstrated that all 46 became fluorescent. When sera from 3 patients with Sjögrens syndrome were utilized, the chromosomes did not become fluorescent even though they had previously been shown to have anti-nuclear factors. Serum from 1 patient with nephrosis resulted in fluorescent staining of the chromosomes while that from another case did not react.

Significance to the Program of the Institute:

The anti-nuclear factors found in serum of patients with the so-called auto-immune diseases are of importance for a better understanding of the mechanisms involved. It may be possible to find sera or fractions of sera which are more specific in their chromosomal reactions.

Proposed Course of Project:

To continue the fluorescent antibody studies on chromosomes utilizing various fractions of serum and to attempt to absorb some of the anti-nuclear activity with nuclear fractions or with nuclei from one species before reacting the sera with chromosomes from another species.

Project Subtitle III: Localization of *Trypanosoma rangeli* in the Insect Host

Objectives:

To determine the organ localization of Trypanosoma rangeli in the arthropod host and to study the developmental relationships between the parasite and the insect vector.

Methods Employed:

Rabbits were immunized with the culture form of T. rangeli and fluorescent labeled conjugates prepared from a globulin fraction of the immune serum. Frozen sections of the insect host, Rhodnius prolixus, (either artificially or naturally infected with the trypanosome) have been prepared and stained with conventional stains. The direct and indirect fluorescent antibody technique has been used in preliminary trials.

Major Findings:

Using conventional stains on frozen sections the trypanosomes have been localized in the salivary glands. Although our results with fluorescent antibody are very preliminary it seems that we may be able to localize the parasite in the salivary glands and perhaps in other organs.

Significance to the Program of the Institute:

The successful localization of T. rangeli in fresh-frozen sections of whole insects opens up the possibility of the precise localization of other infectious agents in arthropod hosts. Detailed studies on the developmental relationships between the parasite and the insect vector are now within the realm of possibility.

Proposed Course of Project:

The fluorescent antibody studies will be continued.

Part B included: Yes X No

Serial No. NIAID - 148

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards and Publications

Publications:

Tobie, J. E., Burgdorfer, W., and Larson, C. L.: Frozen Sections of Arthropods for Histological Studies and Fluorescent Antibody Investigations. Exp. Parasitology. 11:50-55, 1961.

Tobie, J. E., and McCullough, N. B.: Serologic Evidence of Leptospira pomona Infections in Meat Inspectors. J.A.V.M.A. 138: 434-436, 1961.

Milch, Robert Austin, Tobie, John E. and Robinson, Robert A.: A Microscopic Study of Tetracycline Localization in Skeletal Neoplasms. Jour. Histochemistry and Cytochemistry. 9:261-270, 1961.

Krooth, Robert S., Tobie, John E., Tjio, J. H. and Goodman, Howard C.: Reaction of Human Sera with Mammalian Chromosomes Shown by Fluorescent Antibody Technique. Science. 134:284-286, 1961.

Tobie, John E. and Coatney, G. Robert: Fluorescent Antibody Staining of Human Malaria Parasites. Exp. Parasitology. 11:128-132, 1961

Tobie, John E. and Beye, Henry K.: Human Infection With a Subcutaneous Filarial Worm. Am. Jour. Trop. Med. Hyg. In Press.

Kuvin, S. F., Tobie, J. E., Evans, C. B., Coatney, G. Robert and Cantacas, P. G.: Antibody Production in Human Malaria as Determined by the Fluorescent Antibody Technique. Science. In Press.

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 150
1. Laboratory of Immunology
2. Applied Immunology
3. Bethesda, Maryland

Part A:

Project Title: The Relationship of Delayed and Immediate Hypersensitivity to Allergic Thyroiditis in inbred Guinea Pigs.

Principal Investigator: Dr. Philip R. B. McMaster

Other Investigators: None

Cooperating Units:

Dr. Edwin M. Lerner, LGFAR, Serial No. NIAID - 117

Man Years:

Total: 0.2
Professional: 0.2
Other: None

Project Description:

Objectives:

To determine the relative importance of delayed and immediate hypersensitivity to thyroid extract in the etiology and pathogenesis of allergic thyroiditis.

Methods Employed:

Two groups of Strain 13 histocompatible guinea pigs, one immunized with the aid of complete adjuvant, the other with the aid of incomplete adjuvant, were compared with respect to the development of circulating antibody, delayed hypersensitivity to thyroid extract, and thyroiditis.

Major Findings:

A close correlation between the appearance of delayed hypersensitivity to thyroid extract and allergic thyroiditis has been found. Thyroiditis has been produced in some guinea pigs as early as 5 days after immunization. Lesions were found in 100% incidence 16 days after immunization, and at 7 weeks the disease was severe and extensive in all animals. This has persisted as long as 6 months, at which period some decrease in severity has been found. Other animals are being followed as long as lesions continue to be found.

Delayed hypersensitivity to thyroid extract, as determined by skin test, has been noted in most animals at 5 days after immunization, and

has been consistently associated with the finding of thyroiditis, at both the 5 day and 1 week stages. Some decrease in skin test hypersensitivity has been found at 6 months.

The portion of this project involving the transfer of intact lymph node cells is being conducted as a collaborative effort as described in Project Serial No. NIAID - 145.

Significance to The Program of the Institute:

As a result of these studies, a close correlation has been demonstrated between delayed hypersensitivity to the thyroid and experimental allergic thyroiditis. This, therefore, provides experimental evidence for the role of delayed hypersensitivity in the pathogenesis of experimental allergic thyroiditis and suggests the possibility of a similar role in the development of allergic thyroiditis in humans.

Proposed Course of Project:

The long term course of this disease in guinea pigs is now being followed in preparation for a study of the effects of desensitization to an auto-antigen upon an auto-allergic disease, for which this particular condition is most admirably suited.

Part B included: Yes X No

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications:

McMaster, Philip R.B., Lerner, Edwin M. 2nd, Exum, Eurmal D.: The Relationship of Delayed Hypersensitivity and Circulating Antibody To Experimental Allergic Thyroiditis in Inbred Guinea Pigs. The Journal of Exper. Med. 113:611-624, 1961.

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 151
1. Laboratory of Immunology
2. Applied Immunology
3. Paris, France

Part A:

Project Title: The Purification and Characterization of a Proteolytic Enzyme of Human Spleen

Principal Investigator: Dr. Philip R. B. McMaster

Other Investigators: None

Cooperating Units:

Dr. Pierre Grabar, Laboratorie de Chimie Microbienne, Institut Pasteur, Paris, France

Dr. Claude Lapresle and Dr. Tom Webb, Institute Pasteur, Paris, France

Man Years:

Total: 0.5
Professional 0.5
Other: None

Project Description:

Objectives:

To isolate and purify a proteolytic enzyme from human spleen which cuts human albumin into large precipitating fragments in preparation for the isolation of these fragments and comparative analysis of the antigenic structure of these fragments produced by homologous versus heterologous enzymes.

To study the reaction of this enzyme with certain substrates to compare its activity with known cathepsins of animal origin, and to further characterize the enzyme by its electrophoretic mobility.

Methods Employed:

An enzyme has been extracted from human spleen, and purified using DEAE columns, carboxymethyl columns, and sephadex columns. The chromatographic analysis was made by the method of Sober and Peterson, while the mobility was determined by the method of Uriel, and the nature of the activity of this enzyme compared with known cathepsins by the method of Lapresle and Webb. The substrate degradation products have been studied by direct electrophoresis and by immunoelectrophoresis.

Major Findings:

A method of purifying the desired enzyme has been established. That it is a single type of substance has been demonstrated by column chromatography, in addition the electrophoretic mobility and pH activity curve have been determined, and a comparison of the catheptic activity of this enzyme with known cathepsins completed. Exhaustive attempts to determine the Michaelis constant of this enzyme for human albumin have failed. Four large fragments of human albumin resulting from the degradation of the native protein by the human enzyme have been found by immunoelectrophoresis.

Significance to the Program of the Institute:

This study combines several fundamental problems bearing on the nature of antigenicity, and the possible role of enzymes in the degradation of antigens as a primary step in antibody formation. It leads to an examination of the chemical differences between the degradation products of a substrate degraded by homologous versus heterologous enzymes and consequently to the elucidation of the role of enzymatic activity in antigenicity. Information concerning the chemical nature of human albumin and its antigenic sites may also be obtained by these methods.

Proposed Course of Project:

To isolate the different degradation products of human albumin degraded by human enzyme, and to determine the nature of the end groups of each product as well as to determine the quantity of each amino acid in each fragment. The type of bond cut by the enzyme will be measured by the method of Sanger using chain B insulin. These results will be compared with those obtained by Lapresle and Webb using the same substrate degraded by heterologous enzyme.

Part B included: Yes No X

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 152
1. Laboratory of Immunology
2. Applied Immunology
3. Bethesda, Maryland

Part A:

Project Title: The Chemistry of Antibodies

Principal Investigator: Dr. Wilton E. Vannier

Other Investigators: None

Cooperating Units:

Project Subtitle I - Dr. Philip Fireman, LCI

Project Subtitle II - Dr. James Miller of the Department of Infectious Diseases of the University of California, Los Angeles, California

Man Years:

Total: 1.66
Professional: 0.66
Other: 1.0

Project Description:

Project Subtitle I - The Chemistry of the Skin Sensitizing Antibodies of Atopic Human Allergy

Objectives:

To study the physical, immunochemical and biological properties of the skin sensitizing antibodies of sera of individuals with atopic allergies.

Methods Employed:

Sera from suitably selected ragweed sensitive individuals are being fractionated by DEAE-cellulose chromatography and starch block electrophoresis. The fractions are examined by immunoelectrophoresis. The skin sensitizing antibody activities are assayed by passive transfer tests in normal human volunteers.

Major Findings:

A distribution for the skin sensitizing antibody activity after DEAE-cellulose chromatography similar to that reported by others has been found. On starch block electrophoresis (in barbital pH 8.6,

micron=0.1) the antibody activity is concentrated in the fast gamma-globulin or slow beta-globulin region. Various methods of carrying out the passive transfer assay in normal human skin are being studied in order to determine the best method for antibody quantitation.

Significance to the Program of the Institute:

The physical properties of the skin sensitizing antibodies of atopic allergy are of fundamental importance in understanding allergic reactions. The skin sensitizing antibody is qualitatively different from most ordinary precipitating antibody in its electrophoretic and sedimentation properties and in that it is retained locally when injected intradermally rather than diffusing away. The process of antibody fixation to tissues is an important problem in all allergic reactions and any differences that can be demonstrated in chemical or physical properties between ordinary precipitating and skin sensitizing antibody may be important in this process.

Proposed Course of Project:

The work on more precise quantitation of the skin sensitizing antibody will be continued as this is basic to all our other studies. Various conditions for the cellulose chromatography of the active skin sensitizing antibody fraction from starch zone electrophoresis will be explored. It is hoped that with the prior removal of a large amount of inactive protein, by zone electrophoresis, a more efficient and revealing chromatographic fractionation of the skin sensitizing antibodies may be obtained. After the quantitation of the antibody activity is more precisely worked out we plan to study antibody inactivation by various substances and look for the possible presence of antibody activators among serum fractions.

Project Subtitle II - The Chemistry of the Antibodies of Syphilis

Objectives:

To study the physical properties of some of the antibodies associated with syphilitic infection and to study a macroglobulin antibody system in which a specific purification of antibody may possibly be achieved.

Methods Employed:

As a first step sera from individuals with various stages of syphilitic infection are being fractionated by starch block electrophoresis and ultracentrifugation methods and the fractions tested for VDRL flocculation antibody and for TPI antibody.

Major Findings:

Preliminary experiments with human sera from patients with late syphilis have shown a relative concentration of the TPI antibody activity in the more rapidly migrating gamma-globulin fraction as obtained by starch block electrophoresis. This is in accord with previous electrophoresis convection studies of rabbit syphilitic sera.

Significance to the Program of the Institute:

There is confusion in the literature with regards to the interrelationship between the antibodies in syphilis as measured by the various tests available. Studies, such as this, involving the preparation of purified antibody fractions may help to clarify the problem. The study of the chemical and physical properties of specifically purified macroglobulin antibody may yield basic information regarding the structure of these antibodies and their role in immune processes.

Proposed Course of Project:

The work will be continued using the methods indicated and sera from individuals with early and congenital syphilis as well as late syphilis. Active fractions generated by the above methods will be further studied by cellulose ion exchange chromatography. Assay by immunoelectrophoresis to try to correlate the antibody activities with known serum components will be carried out.

Project Subtitle III - The Fractionation and Specific Purification of Antibody

Objectives:

1. To explore possible specific methods of antibody purification.
2. To achieve the chromatographic fractionation of specifically purified antibody and study changes in the distribution of specific bonding affinities of antibodies during the course of immunization.

Methods Employed:

Specific antigen-antibody aggregates are dissociated at low pH and the antibody recovered. Cellulose coupled with p-arsanilic acid through tyramine is being used as a solid adsorbent to purify and fractionate antibody directed against the p-arsanilic acid hapten.

Major Findings:

It has been found that the cellulose adsorbents easily remove antibody from serum but that the complete elution of the antibody from the adsorbent has been difficult. It is hoped that suitable chemical modification of the adsorbents may solve this problem.

Significance to the Program of the Institute:

The general problem of specific purification of antibody is one that is of basic importance for chemical work in immunology. The presence of large amounts of nonspecific protein with antibody complicates or prevents a study of the chemical reactions of antibodies, the structure of antibodies or the physical properties of antibodies. A study of the heterogeneity of antibodies and especially the influence of degree or route of immunization on the distribution of antibodies found will provide additional basic information about the nature of the immune process.

Proposed Course of Project:

The yield, purity and immunochemical characteristics of antibody removed by the specific adsorbents and purified by desorption will be further investigated. The effect of varying the number and type of hapten groups coupled to the specific adsorbent and the number of other charged groups on its effectiveness as an immune adsorbent will be investigated.

Part B included: Yes No X

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 153
1. Laboratory of Immunology
2. Applied Immunology
3. Bethesda, Maryland

Part A:

Project Title: Basic Studies on the Chemical, Physical and Skin Reactive Properties of Extracts of House Dust

Principal Investigator: Dr. Wilton E. Vannier

Other Investigators: None

Cooperating Units:

Dr. Ernest M. Heimlich, a practicing allergist in Los Angeles, has been testing various house dust fractions for skin reactivity in specifically sensitive individuals.

Man Years:

Total: 0.33
Professional: 0.33
Other: None

Project Description:

Objectives:

1. To study the chemical and physical properties of the materials used clinically for the diagnosis and treatment of house dust allergy.

2. To try to identify the components responsible for the specific skin reactivity.

Methods Employed:

1. Various methods of fractionating dust extracts are used such as zone electrophoresis and cellulose chromatography. Chemical analyses and assay for allergen activity are carried out on the products.

2. The effect on the allergen reactivity of chemical and enzymatic modification of the dust fractions is being studied.

Major Findings:

1. Previously it has been shown that the dust allergen fractions consist of a heterogeneous mixture of acidic polysaccharides

containing 5-20% polypeptide. Starch block electrophoresis studies at low pH and in 7M urea have indicated that the polypeptide and polysaccharide components are linked through covalent bonds rather than being present as separate components or linked by ionic bonds or multiple hydrogen bonds.

2. Preliminary studies using ECTEOLA cellulose indicate that this material may be useful for further fractionation of the allergen components.

Significance to the Program of the Institute:

1. About one-third of all allergic individuals give a positive skin reaction to house dust extracts. These extracts have been used for many years in the diagnosis and treatment of house dust allergy. It is of importance to investigate the chemical nature of these materials and to find out what chemical groups are involved in the skin reactions.

2. A rational standardization of the potency of house dust allergen extracts might well be based on a knowledge of the chemical nature of the active materials.

Proposed Course of Project:

The study of chemically modified and enzyme-treated dust fractions will be continued and the use of ion exchange cellulose chromatography to purify the materials will be further explored.

Part B included: Yes X No

Serial No. NIAID - 153

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications:

Vannier, Wilton E. and Campbell, Dan H.: A Starch Block Electrophoresis Study of Aqueous House Dust Extracts. Journal of Allergy 32:36-54, 1961

Serial No. NIAID - 153

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B: Honors, Awards, and Publications

Publications:

Vannier, Wilton E. and Campbell, Dan H.: A Starch Block Electrophoresis Study of Aqueous House Dust Extracts. Journal of Allergy 32:36-54, 1961

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 154
1. Laboratory of Immunology
2. Clinical Immunology
3. Bethesda, Maryland

Part A:

Project Title: Immunocytochemical Studies of the Reactivity of Human Serum Proteins with Human Tissues in Normal and Disease States

Principal Investigator: Dr. Arthur J. L. Strauss

Other Investigators: Dr. Howard C. Goodman

Cooperating Units: None

Man Years:

Total: 0.5
Professional: 0.5
Other: None

Project Description:

Objectives:

To detect and describe protein fractions (especially γ-2, β-2A, and β-2M globulins) from normal and pathologic human sera which react with human tissues, both normal and pathologic.

To determine the range and specificity of such reactivity in normal individuals and in those with systemic diseases.

To characterize such reactive serum factors; describe their electrophoretic mobilities, and determine whether or not such factors react with tissue constituents in classical antibody-antigen relationship.

To attempt to characterize those tissue constituents with which serum proteins might react.

Methods Employed:

The immunofluorescence technique is used to detect the binding of serum proteins to tissues.

Sodium sulfate precipitation, Rivanol precipitation and DEAE cellulose column chromatography are used to prepare purified human serum protein fractions.

Immunoelectrophoresis and simple electrophoresis on agar gel are employed to study electrophoretic mobilities of serum proteins, and to determine the specificity of antisera prepared and directed against serum protein fractions.

To produce antisera possessing unispecific activity against each of several readily separable human serum proteins, sheep and rabbits are currently being immunized with highly purified preparations of such proteins in Freund's adjuvant. Antisera to human serum γ-2 globulin, β-2A and β-2M globulins and albumin are now in preparation.

Major Findings:

During the short period that this project has been in progress, a serum and tissue "library" has been assembled. No major findings can be reported at this time.

Significance to the Program of the Institute:

This study is adjunctive to the studies already underway in the Clinical Immunology Section which deal with the role of immune mechanisms and autoimmune concomitants in human disease states. Many reports have appeared in the literature, describing the reactivity of human serum globulin with human tissue in a variety of experimental procedures. Such reactivities have been noted in systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, thyroiditis, ulcerative colitis, myasthenia gravis, rheumatic carditis, and myocardial infarction. In general, each published study has dealt with a circumscribed disease state, with reactivity demonstrated with one or a limited number of tissues or tissue extracts. The screening of several hundred sera from an even wider variety of disease states against many different tissue substrates with fluorescent antibodies to human γ-2 globulin, β-2A, and β-2M globulins, etc., should provide additional information as to the specificity of these previously reported findings for any given disease and might point up interrelationships among diverse autoimmune phenomena already described. In addition, additional instances of serum-tissue interactions may be revealed in the course of this study.

Proposed Course of Project:

The present human serum and tissue collections will be enlarged. Serum protein fractionation will be continued to obtain purified serum antigens which will be used in the continued production of unispecific antisera for use in fluorescent antibody studies.

Sera will be screened systematically by means of the indirect fluorescent antibody technique against skin, skeletal muscle, cardiac muscle, kidney, liver, spleen, thymus, thyroid, adrenal, gastrointestinal tissues, lungs, smooth muscle, etc., for reactivity over the electrophoretic range of serum proteins with particular emphasis on γ-2, β-2A and β-2M globulins.

Part B included Yes No X

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 155
1. Laboratory of Immunology
2. Immunochemistry
3. Bethesda, Maryland

Part A:

Project Title: Cellular Production of Gamma Globulin; Studies of Rabbit Allotypes Using the Fluorescent Antibody Technique

Principal Investigator: Dr. James Colberg

Other Investigators: Dr. Sheldon Dray

Cooperating Unit: None

Man Years:

Total: 0.5
Professional: 0.5
Other: None

Project Description:

Objectives:

1. To localize the cellular site of formation of gamma globulin allotypes by fluorescein-labeled antibody.

2. To investigate the number of allotypes produced by a single cell in heterozygotes.

3. To study gamma globulin allotype synthesis in neonatal rabbits.

Methods Employed:

1. DEAE cellulose column chromatography; Sephadex chromatography.

2. Conjugation of gamma globulin with fluorescein isothiocyanate.

3. Direct fluorescent staining of frozen tissue sections.

Major Findings:

Fluorescein conjugates specific for allotypes I and II have been prepared; these react specifically with the respective gamma globulins in the tissues. Non-specific staining has been nearly completely eliminated by use of appropriate liver and bone marrow powder.

Significance to the Program of the Institute:

This project by use of the fluorescent antibody method demonstrates

the histological location of specific gamma globulin types and serves to elucidate the immunogenetics of gamma globulin and the fate of transplanted lymph nodes.

Proposed Course of Project:

With the perfection of this technique, antibodies to the other allotypes will be conjugated. Immunofluorescent studies of lymphoid tissue and spleen cell suspensions of heterozygote rabbits will be studied to demonstrate production of allotypes. Neonatal rabbits will be examined to determine the site and time of production of gamma globulin. Studies on homotransplantation of tissues and on immunologic tolerance will be undertaken with this method. Gamma globulin chimeras will be produced in order to induce tolerance to subsequent transplantation of lymphoid tissue and skin grafts.

Part B included: Yes No X

Form No. ORP-2
Nov. 1961

PHS-NIH
Individual Project Report
Calendar Year 1961

Serial No. NIAID - 156
1. Laboratory of Immunology
2. Clinical Immunology
3. Bethesda, Maryland

Part A:

Project Title: The Study of Delayed Hypersensitivity *In Vitro*

Principal Investigator: Dr. Robert R. Carpenter

Other Investigators: Dr. Howard C. Goodman

Cooperating Units: None

Man Years:

Total: 0.5
Professional: 0.4
Other: 0.1

Project Description:

Objectives:

To determine the usefulness of *in vitro* techniques to study delayed hypersensitivity, particularly in relation to the autoimmune disorders.

Methods Employed:

Lymph node and splenic fragments from normal and sensitive animals are explanted in tissue culture in homologous media. The migration of cells from the explants is measured in the presence and in the absence of antigen. Adult inbred guinea pigs are the source of these explants. The pigs are immunized with various tissue extracts in the presence of Freund's adjuvant for study of tissue and tuberculin sensitivity. To date extracts of homologous thyroid and homologous spinal cord have been employed.

Major Findings:

The addition of purified protein derivative of tubercle bacilli (ppd) regularly inhibits macrophage migration from those lymph node and spleen fragments taken from tuberculin sensitive guinea pigs. Cells from normal animals are only slightly inhibited.

Similar inhibition by a saline thyroid extract of tissues cultured from thyroid sensitive guinea pigs, has not been consistenly demonstrated. Thyroglobulin likewise has not proven to be regularly or specifically inhibitory.

Serial No. NIAID - 156

Significance to the Program of the Institute:

This laboratory is engaged in the study of some of the so-called autoimmune diseases. The project described here is undertaken as a part of the program of detection and study of the abnormal immunologic reactions in these states. Recently, there is increasing evidence of the importance of delayed, tuberculin-type, hypersensitivity to tissue products in these illnesses. This technique should provide a much needed, direct, in vitro method to examine this hypothesis. Up to the present, the skin test has been the only means, and a somewhat unsatisfactory one, for evaluating delayed hypersensitivity.

Proposed Course of Project:

Further experiments with delayed sensitivity to tuberculin and simple protein antigens will be conducted to provide a firm basis for interpreting results with this system. We will continue to attempt to characterize the immunologic abnormalities in experimental thyroiditis and encephalitis using this technique, and ultimately we will hope to use the technique in studying delayed sensitivity in human illness.

Part B included: Yes No X

ROCKY MOUNTAIN LABORATORY

Summary.. 1

170 - Office of the Director.................... 8
171 - Rickettsial Infections.................... 9
172 - Q Fever.................................. 14
173 - Selected Zoonoses of Regional Importance.............................. 21
174 - Transmission of Disease Agents by Certain Vectors.......................... 29
175 - Mechanisms of Hypersensitivity and Its Role in Disease...................... 35
176 - Viruses, Virus Components, and Virus Surfaces................................ 43
177 - Immune Prophylaxis of Mycobacterial Infections............................... 49
178 - Immune Prophylaxis of Salmonella Infections............................... 52
179 - Morphological Elements of Micro-organisms in Immunity and Related Phenomena................................ 56
180 - The Encephalitides....................... 61
181 - Colorado Tick Fever...................... 67
182 - Epidemiology of Human Infections of Special Interest......................... 71
183 - Comparative Pathology.................... 74

PHS-NIH
Summary Statement
Rocky Mountain Laboratory
National Institute of Allergy and Infectious Diseases
Hamilton, Montana
Calendar Year 1961

The annual report of the Rocky Mountain Laboratory again is comprised of reports by project rather than by section because of continued multifold collaboration between units in different sections, a healthy characteristic of our program which we find contributes to a good esprit de corps. Reports have been added this year, however, from the re-established Pathology Section, with the return of Dr. W. J. Hadlow, and the newly activated Epidemiology Section, with the assignment of Dr. R. N. Philip.

Other major personnel changes in the scientific staff include: intra-service transfer of Dr. C. L. Larson from the directorship after his 11 years' fruitful promotion of the scientific stature of RML; transfers of Dr. Lauri Luoto and Nurse Officer Mary L. Casey; the working-assignment exchange between Dr. K. C. Milner and Dr. Berndt Malmgren, of the Karolinska Institutet, Stockholm, Sweden; the establishment of a new unit in the Allergy-Immunology section consequent to the appointment of Dr. J. J. Munoz to the staff; and the imminent transfer and retirement of Dr. W. L. Jellison, who, after more than 3 decades of productive and internationally respected research at RML, has been invited to join the staff of the PanAmerican Sanitary Bureau Zoonoses Laboratory at Azul, Argentina.

Extramural cooperation has continued with personnel of NIH, Bethesda, CDC, MARU in Panama, Arctic Health Research Center, NAMRU-3 in Egypt, Gorgas Memorial Laboratory, Trinidad Virus Laboratory, universities of Minnesota, Georgia, Montana, Utah, Purdue, Saskatchewan, Pasteur Institute of Paris, Karolinska Institutet of Stockholm, and other institutions. Many museums and groups engaged in field work have sought aid from staff specialists in determination of parasites. Because of special capabilities, techniques, or unique facilities developed at RML, 50 professional visitors, 17 of them foreign, have spent or are spending varying amounts of time in conference and study in different sections. Several staff members have also received courtesy appointments to graduate thesis committees of a few universities.

Our investigations have continued with the same general objectives as previously; those of one large group are oriented toward the zoonoses and arthropod-borne diseases, and those of another major group are chiefly concerned with problems related to allergy, immunology, and resistance to disease. Both divisions involve basic laboratory research some of which could lead ultimately to development of applied and control measures. While investigations of various vector-borne illnesses have never actually diminished, occasional changes in emphasis have occurred in the last few years with new leads, such as provided in encephalitis ecology by the mosquito-garter-snake relationship, discovery of new foci of Powassan and California viruses, and

variations in spotted-fever-like isolates. On the other hand, attacks on phases of such nonvector-borne disease problems as tuberculosis, Salmonella, pertussis, poliomyelitis, influenza, and certain mycoses have been intensified in varying degrees.

Tick paralysis. Inbreeding through 4 generations of a paralysis-producing strain of ticks from British Columbia has not produced an increase in the ability to cause paralysis, probably because of our inability to select for the genetic factor in male ticks. However, a new lead on genetic aspects may be the sudden acquisition of paralysis-producing ability by some females resulting from a cross of "paralytic" males with females from our previously "nonparalytic" laboratory stock.

Panama mite studies. To date, no viral agents have been isolated at MARU from many pools of acarines from native fauna. One isolation of Coxiella burnetii (Q fever agent) was accomplished at RML from a new genus of intradermal chiggers off one of the numerous spiny rats. It could not be stated that the host relationship was more than mechanical in this instance. Tests for pathogenic agents in ectoparasites of Panamanian native hosts are continuing.

Tularemia. While it was reported under Pasteurella tularensis last year that "the protection afforded by live organisms was not effective for long periods of time," mice later vaccinated with a living Russian attenuated strain had a high level of immunity for more than 30 weeks.

Further comparison of isolates from different sources and geographical localities has strengthened the belief that a "fully virulent" organism in North America causes severe disease in sheep, hares, rabbits, horses, and man, whereas a less virulent form is common to North America, Europe, and Asia and may require systematic differentiation. Isolates of the latter were obtained or arranged for by Dr. J. F. Bell during part of a Guggenheim Fellowship in Europe.

Diseases of wildlife. Corynebacterium pyogenes was cultured from 3 dead mountain sheep during an epizootic and from 1 deer and 1 elk. These findings suggest that this may be a pathogen of consequence to certain wildlife, as well as to domestic animals.

Three more isolations of rabies from bats this year bring the local total to 24. Contrary to most concepts, rabies can be chronic in mice. A consequent discovery was that recovered mice with limb movement impaired by rabies became heavily infested with ectoparasites. Mechanical interference with combing and grooming, caused by amputating limbs of normal mice, resulted in similar excessive parasite populations. Another unidentified, nonfatal virus was incidentally isolated from bats and passed in tissue cultures and mice.

Serial No. NIAID-170
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Office of the Director

Principal Investigator: C. L. Larson, Director

Other Investigators: C. B. Philip, Acting Director
H. G. Stoenner, Assistant to the Director

Cooperating Units: None

Man Years (calendar year 1961):

Total:	31.5
Professional:	1.5
Other:	30.0

Project Description:

This project involves the following: over-all direction and supervision of the scientific research program of the Rocky Mountain Laboratory; direction of the administrative aspects of the Laboratory including all fiscal, supply, housekeeping, and maintenance activities; providing of scientific services as required by the research staff, including laboratory animal care and breeding, sterile glassware and media preparation, special shop services, library, graphic arts, and adjustment of needs for space, technical help, and field and meeting travel. The duties of Director C. L. Larson at the Rocky Mountain Laboratory were terminated on October 15, 1961, after his return from a year's assignment to the Pasteur Institute, Paris, France.

Part B included: No

Q fever. Field investigations this year were oriented toward a more objective evaluation of the potential public health problem associated with the vast reservoir of infection among dairy cattle. As determined through continuous surveillance, even minor illness among persons exposed to infected dairy cattle could not be attributed to Q fever. However, 87% of persons exposed to infected herds have acquired antibodies detectable by the mouse-neutralization test and the radioisotope precipitation test.

Investigations on developing a safe method of vaccinating humans with conventional Q fever vaccine were continued at Montana State Prison. Most of the skin-test negative volunteers (97%) who last year received from 1 to 3 doses of 10 complement-fixing (CF) units of antigen subcutaneously reacted positively on a skin test given 10 months after vaccination. However, the agglutinin response among groups of vaccinees varied from 45 to 91% and was directly proportional to the number of doses of vaccine received. Studies on a comparable group of volunteers who received from 1 to 3 doses of 1 CF unit of antigen intradermally are not complete, but results to date are similar to those obtained when vaccine was given subcutaneously. Although the resistance induced in human volunteers could not be challenged, vaccination appears to have been effective, as judged by available immunologic and serologic data.

Significant progress has been made in investigations directed toward fractionating C. burnetii into its component antigens. The separation of nontoxic immunogenic fractions from those responsible for inducing or eliciting the hypersensitive state remains a major objective. All antigens of any consequence were determined to be located in the cell wall. Chemical extraction of whole phase I rickettsias with dimethyl acetamide, dimethyl sulfoxide, or a combination of trichloroacetic acid and phenol have yielded protective antigens whose toxicity and ability to induce an allergic response were decreased about 100-fold. Similar extracts of phase II organisms were nonimmunogenic and, in preliminary comparisons of whole phase I and II rickettsias, phase I organisms were more potent vaccines.

Rocky Mountain spotted fever. Because of the results of recent studies on rickettsial variants and observations made possible by fluorescent microscopy, interest in the biology of spotted fever rickettsias has been rekindled. Particular attention has been given to the elucidation of factors which affect the natural infection rate among ticks and to the characterization of spotted fever antigen in naturally infected ticks. Of ticks collected in nature, up to 25% contain specific antigen detectable by fluorescent microscopy but only 1 to 3% contain pathogenic rickettsias.

The ecology of R. rickettsii appeared to be the same in 2 local areas which had similar natural features. Heaviest infestations of immature Dermacentor andersoni occurred on golden-mantled ground squirrels and chipmunks, and virulent organisms were isolated from these rodents for the first time west of the Mississippi.

Heretofore, 4 variant types of R. rickettsii in D. andersoni have been recognized. The spectrum of pathogenicity of these types varies from an ability to cause overt disease and death to a limited ability to cause only

immunizing, inapparent infections. From ticks collected in eastern Montana, many isolates of a fifth type have been recovered. These strains are nonpathogenic for guinea pigs and mice and appear to be antigenically different from other types of R. rickettsii. The relationship of the fifth type to the ecology of spotted fever rickettsia remains to be clarified.

By the use of the fluorescent antibody technique, the gross discrepancy between the incidence of pathogenic rickettsias among D. andersoni and the prevalence of specific antigen in this tick has been tentatively explained by the demonstration of a rickettsialike organism that is noninfectious for chick embryos as well as for guinea pigs. This agent behaves like R. rickettsii because it is transovarially transmitted by D. andersoni, has a similar distribution in tick tissues, and is indistinguishable from R. rickettsii by fluorescent microscopy. Studies will be continued to determine whether this organism may influence the prevalence of pathogenic rickettsias among ticks in nature.

Mechanisms of allergic phenomena. A particular skin protein which had been altered by combination with a simple chemical was determined to play an important role in contact allergy. When a conjugate of a purified heterologous protein and hapten was used, antibody but not contact hypersensitivity to the hapten developed. However, when a conjugate of a simple chemical and a soluble fraction from guinea pig skin was used as a sensitizing agent, contact hypersensitivity to the protein developed. This clearly shows that contact hypersensitivity, such as poison ivy rash, is dependent upon combination of chemicals with substances in the skin.

Since the delayed hypersensitive state can not be quantitated by conventional tests in the skin of sensitized animals, an attempt was made to use the cornea as a site for measuring response to the antigen because this tissue is not vascularized. In animals sensitized with a conjugate of protein and hapten, the reactions in the cornea occurred only to the protein moiety, although circulating antibody to the hapten and associated allergic reactions could be demonstrated in other tissues. In the corneal response, which is primarily polymorphonuclear, leucocytes from surrounding tissue and blood vessels were chemotactically attracted to the antigen.

Although delayed shock is thought to be a systemic manifestation of delayed hypersensitivity, the reaction apparently is dependent also upon circulating antibody. In guinea pigs sensitized with hapten-protein conjugates, only injection of the protein causes the systemic delayed reaction when only delayed hypersensitivity to the conjugate is present. However, after antibodies have appeared, intraperitoneal injection of the conjugate causes a brief initial hyperthermia followed by a distinct hypothermia and lymphopenia typical of delayed shock. At this time delayed shock can not be precipitated by the injection of protein alone but it can be produced by injection of hapten combined with a heterologous protein.

Although many immunologic reactions seen in comparative studies of allergic phenomena in newborn and adult guinea pigs were similar, the delayed response evoked by intradermal injection of antigen could not be elicited

before 14 days after birth. This unresponsiveness is attributed to the inability to excite a normal inflammatory response, but the basis for this inertia has not been fully clarified.

To date, these and related studies on tolerance and the effect of hormones and vitamins on delayed allergy support the thesis that delayed hypersensitivity and antibody formation are but phases of the same process. Fundamental knowledge of the mechanisms involved should provide some insight into the study of allergic phenomena associated with infectious and autoimmune diseases.

Endotoxins. Although the exact chemical composition of endotoxin has not been defined, the biological activity of endotoxin complexes, contrary to previous conceptions, cannot be associated with the lipid fractions, and the active principle is probably a polysaccharide.

In further studies, in which endotoxin complexes were depolymerized by treatment with dilute acid, biological activity, including pyrogenicity, tumor damage, lethality, and immunogenicity, was associated with the size of the molecule. When depolymerization had progressed to the extent that the haptenic units were about 1/100th the size of the original endotoxins, all biological activity was eliminated. These studies indicate that one of the major requirements for endotoxin to elicit host reactions is a macromolecular complex of critical size.

Other methods of depolymerizing and recombining the haptenic units will be used to clarify the relationship between molecular size and biologic activity.

Morphological elements of microorganisms. Through collaboration with designers and engineers of a firm specializing in the manufacture of laboratory instruments, a self-contained refrigerated cell-fractionater patterned after the prototype made at RML was tested and developed for the market. The machine is designed so that biological materials can be subjected to any selected pressure up to 60,000 psi and then by sudden release of material through a cooled orifice, organisms or tissue cells can be disintegrated without denaturation of any of the cellular components. During the past year, 12 guest workers and visitors came to RML specifically for the purpose of using this instrument or learning more about it. Since cellular material, varying from the size of small bacteria to large mammalian cells, can be completely disintegrated without denaturing any of the chemical constituents, this instrument will have varied and extensive application in diverse problems in bio-medical research.

Viruses. The development and application of a radioisotope precipitation technique (RIP test) to serology of virus diseases and the study of virus synthesis received major consideration. This technique is based on the principle that radioactive particulate antigen (25 mμ to 300 mμ in diameter) sensitized by specific antibody is agglutinated after species-homologous antiglobulin is added to the reaction. No removal of excess globulin is necessary as it is in conventional Coombs type tests where visible precipitate, rather

than removal of radioactivity from suspension, is the indicator. The test has been standardized so that it possesses a high degree of sensitivity and specificity. In the determination of specific antibody, the test is 30-fold more sensitive than the tissue-culture neutralization test for poliovirus and 100-fold more sensitive than agglutination or complement-fixation tests for Q fever. By the use of an inhibition-type modification, it is possible to detect as little as 0.1 to 0.001 μg of antigen. The inhibition-type modification has been found particularly useful in studies of viral metabolism wherein it is necessary to detect specific viruses, viral components, and enzymes which may be stimulated by RNA and DNA of plant and animal viruses.

Immunoprophylaxis of tuberculosis. Heretofore, resistance in mice against challenge with virulent tubercle bacilli could be induced only by vaccination with living virulent organisms and not with killed organisms or fractions thereof. However, recent studies of the immunogenicity of phenol-extracted young cells are extremely impressive. Guinea pigs and rabbits inoculated intradermally with these cells do not develop local lesions or delayed hypersensitivity but they do develop antibodies. In preliminary experiments, mice vaccinated with phenol-extracted 4-day-old cultures of BCG were resistant to subsequent pulmonary infection with virulent organisms. If further studies confirm the nature of this immunologic response in laboratory animals, these findings may have a profound influence on the control of tuberculosis in this country and abroad.

Publications. Progress in reporting the results of our research is comparable to that of last year's Annual Report when the staff was credited with 48 published papers and 26 in press. Of 70 accepted papers so far this year, 34 have been published.

Comment. It is a satisfaction to note that a substantial number of the administrative problems posed in last year's comments have been ameliorated or are in a firmed position. With the acquisition in recent years of a number of outstanding young scientists in different units, however, the need for an over-all reassessment of our future objectives and research aims is recognized, and an effective way to meet this need is under consideration.

A big boost to the efficiency of our production and needed space readjustment will be the new outside experimental animal and insectary facilities, an outside shop which will permit expansion of the library and an adequate conference room, a small outside facility for storage of flammables, and other internal renovation made possible by a congressional appropriation of $750,000. Plans are now in the preliminary stages. A nearly completed "biogen room," which has special safety features, will greatly facilitate mass production of "hot" antigens, as well as minimize the present Q fever hazard to visitors.

Cornelius B. Philip
Acting Director

In the absence of a usable skin test for detecting the protozoon, Toxoplasma microti in Microtus, an effective method that does not cause permanent injury to the animal was devised for observing brain lesions through a slit in the scalps of mice. Tolerance for this organism may result from *in utero*, or very early postnatal, infection in native mice.

A fungus, Emmonsia crescens, found in certain indigenous small animals of several continents, may also infect man, as indicated by positive passive cutaneous anaphylaxis tests applied to human serums.

Ar-bo viruses. WEE virus from Culex tarsalis at Vale, Oregon, was isolated during the midsummers of 1960 and 1961, but not in the intervening months from October to May or from bird tissues collected during this period. These findings suggest that latent infection in birds during the summer months is unlikely. The overwintering virus mechanism still remains illusive, though recovery of virus in the spring of 1961 from captive garter snakes that had been bitten before hibernation by infected mosquitoes in the fall of 1960 is suggestive, in spite of failure, as yet, to show natural infection in such snakes. This lead has been a popular one and has caused examination of reptile-virus relationships in many countries.

Three isolates of California virus have been made in the Bitterroot Valley from ticks off a snowshoe hare, a golden-mantled ground squirrel, and a chipmunk. Antibodies occurred in a low percentage of these local animals. This naturally leads to an inquiry into the role of ticks as vectors.

An additional isolation of Powassan virus from ticks in western South Dakota has resulted in intensified ecological studies in this area with consequent finding of antibodies in serums from local chipmunks and wild mice. A human serum from western Minnesota and one from central North Dakota (of 1,058 miscellaneous samples tested) showed high neutralizing indexes against this virus. Regional field work will be focused on ecologic and public health aspects of this virus, in view of the known significance of the related Russian spring-summer encephalitis.

Epidemiology. Haemagglutination-inhibition tests on serums of Alaskan natives of all ages in villages with and without histories of attack during the 1918-19 pandemic of influenza have revealed good correlation between A/swine antibodies in persons living at that time and the historic occurrence of disease.

Corneal scarring among some 40% of Eskimo children in southwestern Alaska might be related in some manner to tuberculosis, as suggested by correlation of lesions with tuberculin skin sensitivity.

In epidemiologic investigations, which are somewhat opportunistic in nature and are made in collaboration with state health organizations and western physicians, basic aspects of diseases of primary interest to our program will be stressed rather than surveillance or applied epidemiology.

Serial No. NIAID-171
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Rickettsial infections

Principal Investigator: C. B. Philip

Other Investigators: E. J. Bell, W. Burgdorfer, C. M. Clifford, G. M. Kohls, D. B. Lackman, C. L. Larson, R. A. Ormsbee, and H. G. Stoenner

Cooperating Units: None

Man Years (calendar year 1961):

Total:	11.0
Professional:	3.0
Other:	8.0

Project Description:

Objectives:

These studies are directed toward obtaining a better understanding of the natural history of Rocky Mountain spotted fever. Particular attention has been given to the ecologic factors which affect the maintenance of Rickettsia rickettsii in nature, and to those which determine the kinds of variant strains of that organism occurring in tick populations of different localities.

Methods:

Field studies were conducted in different localities to determine (a) infection rates in the tick populations, (b) major animal hosts of the immature ticks, (c) the incidence of new infections among these hosts during the tick season, and (d) the kinds of variant rickettsial strains present in the various local tick populations. In the laboratory, determinations of infection in ticks and of past infection in mammalian hosts were made by means of the fluorescent antibody and complement-fixation tests, respectively.

Part B included: Yes

Major findings:

1. The ecology of R. rickettsii appeared to be the same in two Bitterroot localities which exhibited similar natural features. Thus, in both areas, the infection rate of adult ticks, as determined by fluorescent microscopy, was found to be about 20%; predominant local animals supporting immature Dermacentor andersoni were the golden-mantled ground squirrels, chipmunks, and wood rats; and specific complement-fixation antibodies against R. rickettsii were found in the sera of a high percentage of ground squirrels and chipmunks but not in sera of wood rats.

2. Isolation of R. rickettsii was made for the first time from two golden-mantled ground squirrels and one chipmunk trapped in the study areas.

3. Quantitative studies on transovarial transmission in D. andersoni were continued this year. It was found by fluorescent microscopy that 43 experimentally and 5 naturally infected ticks passed R. rickettsii to 100 per cent of their progenies. The biological properties of the rickettsia did not change during the entire life cycle of the offspring.

4. A rickettsia-like organism was demonstrated by the fluorescent antibody technique in approximately 15 to 25 per cent of adult D. andersoni collected in nature. This agent behaved like R. rickettsii in that it is transmitted transovarially by D. andersoni, has a similar distribution in the tick tissues, and is indistinquishable from R. rickettsii by the fluorescent antibody test. However, the organism appears to be noninfectious for chick embryos as well as for guinea pigs.

5. Several agents which were consistently nonpathogenic for guinea pigs and mice and which appeared to be antigenically different from the known variants of R. rickettsii were isolated from ticks. These isolates grew readily in embryonated chick eggs, and infected yolk sacs of the 10th and later serial egg passages had titers of between 10^4 and 10^5 egg LD_{50}. When such cultures were tested as antigens in the complement-fixation test, they reacted specifically with antisera against members of the spotted fever group of rickettsias. Furthermore, large doses of the isolates immunized guinea pigs against subsequent infection with R. rickettsii. However, such immunized animals developed little, if any, serum neutralizing antibodies against the toxin of R. rickettsii. Hence, they appeared to be antigenically different from the latter agent.

Significance:

The recovery of 3 strains of R. rickettsii from naturally infected golden-mantled ground squirrels and chipmunks provides evidence that these animal hosts of D. andersoni are important in the natural maintenance of Rocky Mountain spotted fever. This is the first time this

agent has been isolated from wild animals caught west of the Mississippi.

The observation that infected D. andersoni passed R. rickettsii to 100% of their progeny suggested that a relatively high percentage of this tick in nature should be infected. However, when the infectivity rate of such ticks was determined it was found to be extremely low (1% to 3%). The reasons for this discrepancy are not clear. It may be speculated that under certain ecological circumstances R. rickettsii becomes noninfectious for embryonated chick eggs. In support of this hypothesis is the demonstration of an organism in D. andersoni which behaves like R. rickettsii but which, nevertheless, could not be isolated in eggs or in guinea pigs.

Further studies are required to obtain unequivocal evidence that the nonpathogenic rickettsia isolated from D. andersoni is antigenically different from R. rickettsii. If so, as present evidence indicates, then the number of distinguishable strains of rickettsia-like agents infecting this tick in nature will be increased to five. This fact should be of particular advantage to studies of rickettsial variation or of the ecology of R. rickettsii.

Proposed course:

Testing of material collected this year will be continued. It is planned to initiate similar ecologic studies in new areas which show different biotic characteristics than those previously investigated. Such differences already known to exist in these areas pertain to the species of the major hosts of immature ticks, percentage of the tick population infected, or kind of strain of R. rickettsii present in the ticks.

Studies on the transovarial transmission of R. rickettsii in D. andersoni originally infected in nature will be continued for purposes of determining the rate of hereditarily acquired infection in successive generations, and of ascertaining whether continuous transovarial passage of this agent results in changes in its biological properties. Initial studies on the properties of the nonpathogenic rickettsia from ticks will be elaborated.

Serial No. NIAID-171

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Burgdorfer, W.: Evaluation of the fluorescent antibody technique for the detection of Rocky Mountain spotted fever rickettsiae in various tissues. Path. et Microbiol. 24(Supplement): 27-39. 1961.

Larson, C. L.: Some aspects of research on rickettsial diseases at the Rocky Mountain Laboratory. Bull. de la Société de Path. Exotique. 54(2): 169-173. Mar.-Apr. 1961.

In press:

Bell, E. J. and Stoenner, H. G.: Spotted fever vaccine. Potency assay by direct challenge of vaccinated mice with toxin of Rickettsia rickettsii. J. Immunol.

Kohls, G. M.: Rocky Mountain spotted fever. History of Medical Department of the Army in World War II.

Philip, C. B.: Scrub typhus. History of Medical Department of the Army in World War II.

Stoenner, H. G., Lackman, D. B., and Bell, E. J.: Factors affecting the growth of rickettsias of the spotted fever group in fertile hens' eggs. J. Inf. Dis.

Eklund, C. M., Stoenner, H. G., and Kohls, G. M.: Rocky Mountain Spotted Fever. Tice's Practice of Medicine.

Honors and Awards relating to this project:

Dr. C. B. Philip

Invited to give lecture on rickettsias for class in microbiology at Montana State University, Missoula.

Serial No. NIAID-171

Dr. W. Burgdorfer, Dr. R. A. Ormsbee, and G. M. Kohls

Invited to attend the Rickettsial Diseases Conference in Washington, D. C. By invitation, Dr. Burgdorfer presented a paper on "Evaluation of the fluorescent antibody technique for the detection of Rocky Mountain spotted fever rickettsiae in various tissues," and Mr. Kohls one on "New Guinea mites in relation to scrub typhus." The latter was given at a Symposium on Scrub Typhus held in conjunction with the Conference.

Serial No. NIAID-172
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Q fever

Principal Investigator: H. G. Stoenner

Other Investigators: R. L. Anacker, M. L. Casey, R. K. Gerloff, W. T. Haskins, B. H. Hoyer, D. B. Lackman, L. Luoto, R. A. Ormsbee, E. G. Pickens, and E. Ribi

Cooperating Units: Montana State Board of Health, Idaho State Dept. of Health (Dr. J. A. Mather and W. W. Benson)

Man Years (calendar year 1961):

Total:	16.0
Professional:	5.0
Other:	11.0

Project Description:

Objectives:

A changing epidemiologic picture has been associated with the recent increase in prevalence of Coxiella burnetii infections in livestock in the United States. Therefore, studies were made to explain the dearth of recognized clinical cases and to develop more sensitive serologic techniques which would detect the total immune response in man and animals. Related studies were directed toward improving the potency and reducing the toxicity of vaccines, and developing practical and safe immunizing procedures. The chemical and immunologic relationships between phase I and phase II rickettsias also were investigated. Epidemiologic investigations and laboratory studies will be described separately.

A. Epidemiologic investigations

Methods:

Controlled epidemiologic studies and surveillance programs, supported by a variety of laboratory tests designed to measure the immunologic experience of man and livestock, were utilized. Studies directed toward the development of a safe immunization program were continued.

Part B included: Yes

Presently, most rural people exposed to infected dairy herds experience immunizing infections without developing clinical illness. Conceivably, the current pattern is attributable to a change in virulence of the organism rather than any change in the innate resistance to man.

The isolation of C. burnetii from mammals and their ectoparasites in the Bitterroot Valley again illustrates the broad facets and complex nature of the ecology of the organism. Apparently, the organism is capable of maintaining itself in separate cycles in nature and in domestic animals.

With Q fever vaccines presently available, it is possible to immunize skin-test negative persons without inducing untoward reactions. Results of recent studies on fractions of C. burnetii suggest that vaccines may be improved by separating skin-reactive substances from those which are immunogenic. Because this improved vaccine could be used in persons regardless of their state of hypersensitivity, such a product would be extremely useful if any extensive immunization program became necessary for national defense.

Proposed course:

Although acute disease in man has not been associated with the recent extension of the reservoirs of infection in livestock, chronic disease which may be attributable to C. burnetii has not been evaluated to any extent. Recovered patients and seropositive persons will be studied for evidence of cardiovascular disease, abortion, and other sequelae.

B. Laboratory studies

Methods:

In continued investigations on the cellular constituents of C. burnetii, fractions obtained by chemical and physical means were assayed for CF activity, immunogenic and toxic properties in normal rabbits and for allergy-exciting properties in sensitized rabbits. Serologic techniques were modified and new tests were developed to improve the detection of various antibodies to C. burnetii.

Major findings:

1. When trichloroacetic acid (TCA) extracts of phase I organisms were compared on a weight basis with whole cells, TCA extracts were more reactive in the CF test and were 1/10th as immunogenic but only 1/100th as active in causing skin reactions in sensitized rabbits. Further treatment of TCA extracts with phenol increased the CF activity, reduced the nitrogen content about one-half, and reduced the activity of the substance in the skin of sensitized rabbits at least 10-fold.

2. When used as vaccines in guinea pigs, dimethyl sulfoxide (DMS) and dimethyl acetamide (DMAC) extracts of phase I C. burnetii behave as potent protective antigens whose toxicity and ability to induce an allergic response have been decreased about 100-fold. Similar extracts of phase II organisms were nonimmunogenic and, in preliminary comparisons of the immunogenicity of whole phase I and II rickettsias, phase I organisms were significantly more effective.

3. When the immunogenicity of fractions of C. burnetii was determined in guinea pigs, the presence of CF antibodies could not be correlated with immunity. In contrast, immunity could be correlated with phase I agglutinins measured by the agglutination-resuspension test. In this test the antigen, which consists of 200 µg of purified phase I C. burnetii/ml, is mixed with serum dilutions; the mixture is incubated at 37°C for 30 minutes and then centrifuged at 2,000 g for 10 minutes. If agglutinins are present, the pellet breaks up into large aggregates when tubes are shaken or agitated.

4. Studies on the mechanisms involved in the radioisotope precipitation (RIP) test revealed that large particles, such as rickettsias, behaved essentially like poliovirus in complexing with specific antibody and antiglobulin. Thus, the technique could be applied to the detection of antibodies against C. burnetii. Upon further exploration, the test, which was determined to be extremely sensitive and specific, was successfully used for evaluating the immune response in vaccinated persons and for determining the total immunologic experience of a population exposed naturally to Q fever rickettsias.

In the RIP test, radioactive purified antigen is mixed with the serum and the mixture is incubated for 1 hour at 37°C. After antiglobulin is added, the test is incubated for another hour at 37°C and then held for 1 to 72 hours at 4°C. After low-speed centrifugation, the presence of specific antibody is determined by counting radioactivity in the sediment and supernatant.

The sensitivity of the test is indicated by the following findings: of 33 serums from vaccinees, antibodies were detected in 2 by CF test, in 11 by the CA test, in 22 by the agglutination-resuspension test, in 29 by mouse-neutralization test, and in 28 by RIP test. Agreement between the last 2 tests was almost complete. In Ravalli County, where Q fever is prevalent among dairy cattle, 87% of persons exposed to infected herds were seropositive, whereas in other areas of the state essentially free of bovine Q fever only 4% of the general population were seropositive.

5. In further studies to clarify the relationship between phase I and phase II C. burnetii, the rate of phase conversion has been studied in tissue culture. Whereas up to 20 egg passages may be required to convert phase I to II, essentially complete conversion in agar tissue culture occurred in 2 passages.

Significance:

Because of the number of serologic techniques available, the complexity of the immune response in man and animals, and the changes in antigenic structure associated with growth of the organism in various tissues, the serodiagnosis of Q fever has been confused. Correlation between techniques often has been poor because the tests vary in sensitivity and each may not measure the same antibody.

Heretofore, epidemiologic studies have been handicapped because few tests measured the total immunologic response in an exposed population. This now appears possible in view of the extreme sensitivity of the recently developed RIP test and its excellent correlation with the mouse-neutralization test.

The true relationship between phase I and II Q fever rickettsias is still unexplained. Because of their varying behavior in serologic systems and their divergent immunogenic properties, significant differences in antigenic composition are suggested. This thesis is corroborated by the finding that dimethyl sulfoxide fractions of phase I and II organisms differ in immunogenic potency. Further studies of the chemical components of Q fever rickettsias and their associated biologic properties should help to explain the exact differences between phase I and phase II organisms.

Proposed course:

In studies on the improvements of vaccines, efforts will be directed toward the separation of immunogenic fractions from associated components that induce or elicit hypersensitivity. Attempts will be made to clarify the relationship between phase I and phase II organisms and the factors responsible for phase conversion.

Serial No. NIAID-172

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Pickens, E. G. and Gaon, J. A.: Growth of Coxiella burnetii in agar tissue culture. Am. J. Trop. Med. & Hyg. 10(1): 49-52. Jan. 1961.

Stoenner, H. G., Lackman, D. B., Benson, W. W., Mather, J., Casey, M., and Harvey, K. A.: The role of dairy cattle in the epidemiology of Q fever in Idaho. J. Inf. Dis. 109(1): 90-97. July-Aug. 1961.

Luoto, L. and Pickens, E. G.: A résumé of recent research seeking to define the Q fever problem. Am. J. Hyg. 74(1): 43-49. July 1961.

In press:

Lackman, D. B., Frommhagen, L. H., Jensen, F. W., and Lennette, E. H.: Q fever studies. XXIII. Antibody patterns against Coxiella burnetii. Am. J. Hyg.

Lackman, D. B., Bell, E. J., Bell, J. F., and Pickens, E. G.: Intradermal sensitivity testing in man with a purified vaccine for Q fever and notes on preparation and standardization of the vaccine. Am. J. Pub. Health.

Ormsbee, R. A., Bell, E. J., and Lackman, D. B.: Antigens of Coxiella burnetii. I. Extraction of antigens with nonaqueous organic solvents. J. Immunol.

Ormsbee, R. A.: A method of purifying Coxiella burnetii and other pathogenic rickettsiae. J. Immunol.

Honors and Awards relating to this project:

Dr. R. L. Anacker

Invited to participate as a lecturer in the high school science program sponsored by the Montana Academy of Sciences.

Miss M. L. Casey

Invited to take part in discussions in Helena and Billings, Montana, at CDC's course on The Principles of Epidemiology.

Dr. Lauri Luoto

Invited to give talk on Q fever at meetings of Montana Medical Technicians Association, Great Falls.

Invited to give talk on Q fever at the annual meeting of the Canadian Public Health Association, Regina, Canada.

Dr. H. G. Stoenner

Invited to give talk on Q fever at Montana State University, Missoula.

Appointed chairman of the Research Committee of the Intermountain Veterinary Medical Association.

Invited to present talks in Helena and Billings, Montana, at CDC's course on The Principles of Epidemiology.

Serial No. NIAID-173
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Selected zoonoses of regional importance

Principal Investigator: J. F. Bell

Other Investigators: E. J. Bell, W. Burgdorfer, W. J. Hadlow, W. L. Jellison, D. B. Lackman, C. R. Owen, and R. N. Philip

Cooperating Units: None

Man Years (calendar year 1961):

Total:	13.5
Professional:	4.5
Other:	9.0

Project Description:

Objectives:

Elucidation of the ecology of zoonoses (except rickettsioses) is the principal goal of this project. However, special problems such as pathogenesis, prevention, and immunity are pursued in the laboratory.

Methods:

Field work in ecology and laboratory techniques in microbiology, entomology, immunology, and pathology are employed as required for the various studies of this project.

Major findings:

1. Tularemia.

(a) Long-term studies on the duration of immunity following vaccination with viable (Russian) tularemia vaccine and with nonviable (ether-extracted) vaccine demonstrated the superiority of the former. Resistance declined markedly in 30 weeks following administration of nonviable vaccine but remained at a high level after vaccination with viable organisms.

Part B included: Yes

(b) Results of continued studies on correlation of virulence with animal host and with geographic source are supporting the concept of two distinct levels of virulence of the tularemia organisms found in nature, i.e., (1) a "fully" virulent organism found only in North America and capable of causing severe disease in sheep, hares, rabbits, horses, and man, and (2) a less virulent organism common to North America, Europe, and Asia. The less virulent organism also differs in that it does not ferment glycerol.

(c) The failure of serial passage in animals, either in nature or by artificial means, to enhance the virulence of Pasteurella tularensis has been demonstrated. Preliminary experiments indicate, however, that contagiosity may characterize certain organisms independently of virulence.

(d) Experiments on survival of P. tularensis indicate that viability and virulence are well maintained in natural water at 4°C. Freezing the water or raising the temperature to 20°C causes decrease in viability. Buffers of several pH values, and salt solutions, also cause loss of viability. When the organisms are held in nutrient broth, however, viability is maintained but virulence is lost.

(e) Natural limitations to spread of arthropod-borne infections. A renewal of studies on this subject furnished new evidence that (1) tularemia is not usually maintained by transovarial passage and (2) ingestion of blood of an immune animal by a tick may be inhibitory to infection in the tick.

(f) The relationship between P. tularensis and P. novicida is still under study. Good cross protection is afforded by living vaccines of the two organisms. Results of agar gel diffusion tests are inconclusive, but passive cutaneous anaphylaxis tests remain highly specific with absorbed serums.

A field trip for the purpose of reisolating P. novicida at the site of original isolation yielded 4 isolates of another unidentified Pasteurella instead. New studies on tick transmission of P. novicida will be undertaken.

2. Corynebacterium pyogenes cultures were obtained from 3 of 5 mountain sheep that died during an epizootic and from 1 deer and 1 elk.

3. Influenza virus has not been isolated at RML this year.

4. Rabies.

(a) Three isolates have been obtained from bats this year. The total number of isolates from this small area of western Montana now numbers 24. Recent tests have demonstrated that virus may be present

throughout the body at certain stages of the disease.

Very good success has now been attained in maintaining bats in captivity. We have therefore been able to infect bats by intramuscular injection and to observe the results of infection. The results, however, have been confused by an intercurrent, indigenous, but nonfatal infection along with the rabies infection. This virus is also present in salivary glands of the bats, grows in tissue cultures of the salivary glands, and is infectious for mice. Several other viruses isolated in previous years from tissue culture of salivary glands are not infectious for mice. We have also isolated several apparently unrelated viruses from bats and have them under study for identification.

(b) Emphasis has been on presence of live rabies virus in the central nervous system during chronic illness and on rate of development of humoral antibodies. Virus has been isolated as long as 10 days after onset, but humoral antibody has not yet been detected at that time. However, studies are not yet completed.

5. Lousiness in mice as a result of limb disability. Mice that survive infection with rabies virus with serious paralytic sequelae are prone to become heavily infested with lice and mites. The animals thus infested often inflict depilation and lacerations upon themselves. In an effort to determine whether the parasitosis is a consequence of limitation of grooming and whether the self-inflicted injuries are a result of the parasitosis, limbs of newborn mice were amputated to create disability. Amputees kept singly developed tremendous parasite burdens in areas of skin not accessible to grooming. They also developed areas of denudation, but not lacerations, where rubbing of the skin adjacent to the infestation could be done.

6. Microtus studies. Activity has been limited to study of the "M" organism (Toxoplasma microti). The serious obstacle to study occasioned by the inability to recognize the disease in the living animal has been overcome. A technic of slitting the scalp which permits visualization of the brain through the calvarium now permits diagnosis in living animals without permanent harm. Skin tests on infected animals have been found to be negative.

7. Beaver diseases.

(a) Red foot of pen-raised beaver is characterized by redness and erosions of the feet without inflammatory cellular reaction. No pathogenic organisms were isolated.

(b) Subcutaneous abscesses in wild beaver of the upper Flathead Valley, Montana, became very prevalent in the summer of 1961. The disease was characterized by subcutaneous abscesses, emaciation, and splenomegaly. P. tularensis or other pathogens could not be isolated.

Unfortunately, no specimens suitable for pathology were submitted.

8. Adiaspiromycosis (Emmonsia infection). Application of the passive cutaneous anaphylaxis (PCA) test to human serums strongly indicates that man may be infected with Emmonsia crescens.

Significance:

Comparative studies on viable and nonviable vaccines for tularemia in laboratory animals and in naturally exposed animals reveal that viable vaccine is better. Resistance to challenge by organisms of high virulence is greater and of longer duration following vaccination with viable vaccine. Cell-wall vaccine proved worthless in a natural epizootic in beaver kept in pens on a farm.

The classification of the etiologic agent of tularemia is not satisfactory. Data are accumulating to establish the occurrence of an organism characterized by very high virulence that typically infects rabbits, hares, and sheep and is found only in North America and the occurrence of another organism of lesser virulence that is found in water and aquatic mammals and is common to Eurasia as well as to North America. The two organisms are also distinguished by biochemical reactions, but both have some relationship to a third organism, P. novicida, which does not require cystine for growth. Our studies will form a basis for rational classification of these organisms in the genus Francisella Dorofeev and for a better understanding of their ecology.

Repeated isolations of pyogenic Corynebacteria from wild animals with extensive lesions indicate that these organisms may be a serious menace to the natural populations of some species. In most instances the isolations were sporadic, but epizootic illness has been found.

The absence of influenza virus isolations in 1961 reflects the apparent low incidence of the disease in this area.

Findings regarding the "M" organism in voles are of interest inasmuch as lack of reactivity of the tissue around colonies of the organism and lack of reaction to a skin test in infected animals suggest an in utero or very early postnatal infection which induces tolerance in the host.

Results of recent PCA tests strongly suggest that adiaspiromycosis (Emmonsia infection) may be a disease of man as well as of wild animals.

Loss of infection in arthropods after a meal of immune blood could be an important limitation on the spread of infection in nature. Recognition of this phenomenon, when it occurs, is essential to the understanding of ecology of an arthropod-borne disease.

Continued isolation of rabies virus from bats in western Montana and the absence of disease from other species, domestic or wild, are strengthening the evidence for a separate ecology of bat rabies. However, the viruses of bat rabies and of rabies in other species are not distinguishable. Virus has been transmitted to other species by the bite of naturally infected bats, and opportunities for exchange in nature are common. The reason for the apparent separateness is not known.

Persistence of infective virus in the central nervous system of mice with chronic rabies and an absence of humoral antibodies during the critical period of recovery signify that some mechanism other than antibody activity is responsible for survival in rabies. The phenomenon of recovery is very common in mice, and elucidation of the factor responsible is desirable.

Proposed course:

Some of the projects have been terminated or will be terminated soon. All activity in beaver disease work has ceased. The imminent departure of Dr. Jellison will terminate the activities in the field of adiaspiromycosis and on fleas.

Work on tick-transmitted bacterial infections will be increased in an attempt to complete it this year. Other studies on tularemia will be pursued as time permits. One investigator will go to Japan and Korea in order to obtain comparative data and materials from Asia.

Attempts to isolate influenza virus will be made if the disease should occur in the Northwest. Isolations of other pathogens will also be done as indicated. The study of Corynebacterium infections in wild ruminants will be continued.

The newly acquired ability to diagnose "M" organism infection in living voles is a breakthrough which may result in further information on the epizoology and pathogenesis of this disease.

Studies on rabies in bats will be continued at the low level permitted by our facilities. We are greatly handicapped by lack of knowledge of the ecology of the bats and failure of a department of the University of Montana to obtain a grant for study of the epizoology of bat rabies. New viruses isolated incident to this study will be characterized. At least one isolate appears to be unrelated to any of 68 other viruses.

The investigation of the phenomenon of survival with sequelae of mice after rabies infection is nearing the stage of publication, but certain facets will need further study.

Pathogenesis of chronic rabies is being studied in mice and factors probed which are known to alter the immune mechanism in order to determine whether survival can be affected.

New studies will be initiated as proper subjects are encountered and as time permits. In a major new study, emphasis will be placed on development of techniques (especially the CF test) for epidemiologic investigations of diseases caused by the psittacosis-lymphogranuloma group.

Serial No. NIAID-173

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Philip, C. B. and Owen, C. R.: Comments on the nomenclature of the causative agent of tularemia. Internat. Bull. Bact. Nomenclature and Tax. 11(3): 67-72. July 15, 1961.

Jellison, W. L., Drouhet, E., Segretain, G., L'Héritier, M., and Petter, F.: Adiaspiromycose (Haplomycose) chez les mammifères sauvages en France. Ann. Inst. Pasteur 100(6): 747-752. June 1961.

Jellison, W. L., Helminen, M., and Vinson, J. W.: Presence of a pulmonary fungus in rodents in Finland. Ann. Med. Exper. Fenn. 38(4): 1-8. 1960. (Publication not received until 1961).

Jellison, W. L., Vinson, J. W., and Borg, K.: Adiaspiromycosis (Haplomycosis) in Sweden. Acta Veterinaria Scandinavica 2(2): 178-184. 1961.

Jellison, W. L., Owen, C. R., Bell, J. F., and Kohls, G. M.: Tularemia and animal populations: Ecology and epizootiology. Wildlife Disease. No. 17, pp. 1-22. Oct. 1961. (Micro Card)

In press:

Bell, J. F., Moore, G. J., Raymond, G. H., and Tibbs, C. E.: Characteristics of rabies in bats in Montana. Am. J. Pub. Health.

Osgood, S. B., Jellison, W. L., and Kohls, G. M.: An episode of pseudo-pediculosis. J. Parasitol.

Jellison, W. L.: Sodoku. Rat-bite fever due to *Spirillum minus* Carter. Diagnostic Procedures and Reagents, 4th ed. American Public Health Assoc., New York, N. Y.

Owen, C. R., Bell, J. F., Jellison, W. L., Buker, E. O., and Moore, G. J.: Lack of demonstrable enhancement of virulence of *Francisella tularensis* during animal passage. Zoonosis Research.

Larson, C. L.: Tularemia. Diagnostic Procedures and Reagents, 4th ed. American Public Health Assoc., New York, N. Y.

Alexander, A. D., Stoenner, H. G., Wood, G. E., and Byrne, R. J.: *Leptospira naam* (subserotype *dakotii*): A new pathogenic leptospira, not readily cultivated. J. Bact.

Honors and Awards relating to this project:

Dr. J. F. Bell

Awarded Guggenheim Fellowship travel grant to study tularemia in 1961 and 1962 in Europe and Asia.

Dr. W. L. Jellison

Requested to review some material for the new edition of Hull's *Diseases Transmitted from Animals to Man*.

Gave lectures on tick-borne diseases and mammalian taxonomy in relation to disease at the CDC Training Course, Denver, Colorado.

Invited by the PanAmerican Sanitary Board to work on zoonoses in their research laboratory in Azul, Argentina, and plans to leave before the first of the year.

Serial No. NIAID-174
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Transmission of disease agents by certain vectors

Principal Investigator: C. B. Philip

Other Investigators: J. F. Bell, J. M. Brennan, W. Burgdorfer, C. M. Clifford, W. L. Jellison, G. M. Kohls, D. B. Lackman, V. F. Newhouse, and C. E. Yunker

Cooperating Units: MARU, Gorgas Memorial Laboratory (Dr. G. B. Fairchild), U. S. Army (Major V. J. Tipton), NAMRU-3 (Dr. H. Hoogstraal), Norfolk Branch of College of William and Mary (Dr. Daniel Sonenshine)

Man Years (calendar year 1961):

Total:	13.5
Professional:	4.5
Other:	9.0

Project Description:

Objectives:

This group directs its studies toward problems of the intimate relationships between infectious agents and their actual or potential invertebrate vectors. Occasionally, symbiotic organisms in arthropods come under scrutiny, as well as the presumed neurotoxin of tick paralysis in various tick hosts. Facets of the relatively new field of endoparasitic transmission of disease agents are also being developed around a model in the mosquito-borne filariad nematode of the cottontail rabbit.

Methods:

Field and laboratory methods are mainly those developed at the Rocky Mountain Laboratory, including more recent fluorescent microscopy as applied to vertebrate-invertebrate cycles of disease agents.

Major findings:

1. Panama mite project. Tests of acarine parasites (chiggers, parasitoid mites, and ticks) from Panamanian mammals, birds, and reptiles

Part B included: Yes

for agents of disease have so far yielded only one rickettsial and no viral pathogens. Nearly 500 pools of acarines were tested for viral agents in suckling mice at Middle America Research Unit in the first year's work. Subsequently at RML, 43 pools of chiggers (182 original lots) representing many species of 8 genera, including one new species, and 14 pools of ticks (29 lots) were injected into chick eggs, guinea pigs, plus some hamsters and weanling mice. A strain of *Coxiella burnetii* of moderate virulence was isolated from one lot of mites (RML 48489), and confirmed by passage and serology. The mites represented an undescribed genus found intradermally on a common spiny rat, *Proechimys*. Tests of other lots of this mite off the same hosts were either negative or rendered inconclusive by contamination.

2. *Tick paralysis*. Original lines of *Dermacentor andersoni* from British Columbia, inbred for 4 generations, are still showing about the same percentage of positive ticks as reported previously, without hoped-for increase.

The following groups of ticks failed to show experimental transmission: *D. andersoni*, several lots from Vale, Oregon, Wyoming, eastern Montana, and, rather surprisingly, 115 drag ticks from Clinton, western Montana, where a locally acquired tick caused a human case; *D. variabilis*, several lots from eastern Montana, Nebraska, North Dakota, South Dakota, and Minnesota; *D. occidentalis* from southwestern Oregon; and 5 lots of *Amblyomma americanum* from Texas. Tests of *D. albipictus* from local moose and mountain goats were negative or inconclusive.

Though normal RML stock *D. andersoni* have not produced paralysis, when females were mated with BC strain males, some adult progeny became positive. It is hoped further studies may shed light on some explanation, genetic or otherwise.

3. *Taxonomic studies*. In addition to minor collections of mites, ticks, and horseflies received for identification, or other studies in progress, major completed studies published or in press include: chigger mites of Panama and Peru, larval tick differentiation in genus *Argas*, a catalog of Nearctic horseflies, and revisions of Palearctic genus *Nemorius*, and far eastern chrysopine flies. A 10-year supplement (1951-60, inclusive) to the Index of Literature on North American Fleas has been prepared and is now being typed for publication.

Because of their specialties, 2 of the staff have been requested to review and supervise graduate theses at the universities of Utah and Montana, as detailed under honors.

Significance:

Tedious techniques for the harvesting and testing of minute Panamanian mites had to be developed and refined, and at least those

shipped to RML were found difficult to decontaminate for injection into highly sensitive chick embryos. Since the one isolate of the Q fever agent was from intradermal mites, it cannot be stated at present whether this association was biological or mechanical. With more experience in wider areas, it is likely that chigger mites, in view of their widespread importance in the scrub typhus cycle of the Far East, will be found associated with more pathogens.

It still cannot be stated whether the ability of certain ticks to produce tick paralysis involves a genetic factor. The failure to increase the ability by inbreeding is not decisive because only the females can be selected for the genetic factor. The fact that males from positive lines seem to have stimulated the ability in otherwise "nonparalytic" laboratory stocks is suggestive, though the techniques of following this lead will be involved and time-consuming.

Proposed course:

Attempts to recover pathogens from Panamanian acarines will be continued until at least June of next year. In order to attack vector relationships among these and other parasites experimentally, a better balance between field and laboratory problems should be achieved in several RML units, when efforts can be intensified in new insectary facilities now in the initial planning stages.

Attempts will be continued to accentuate the paralysis-producing factor by crossing experiments in certain lines of ticks. When it eventually becomes possible to combine these efforts with controlled simulated variable environmental conditions in the new insectary facilities, the search may be narrowed.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Philip, C. B. and Burgdorfer, W.: Arthropod vectors as reservoirs of microbial disease agents. Ann. Rev. Ent. 6: 391-412. 1961.

Brennan, J. M. and Jones, E. K.: New genera and species of chiggers from Panama (Acarina: Trombiculidae). J. Parasitol. 47(1): 105-124. Feb. 1961.

Brennan, J. M. and Jones, E.: Chiggers of Peru (Acarina: Trombiculidae). Acarologia 3(2): 172-205. April 1961.

Philip, C. B.: *Akamushi* (*Trombidium*) Brumpt, 1910 (Class Acarina): Proposed validation under the plenary powers Z.N.(S.) 400. Bull. Zool. Nomenclature 18(2): 140-142. April 14, 1961.

Philip, C. B.: New North American Tabanidae. XIII. Change of name for a well-known species of *Chrysops*. Ent. News 72(6): 160-162. June 1961.

Kohls, G. M. and Clifford, C. M.: A new species of *Ixodes* (*Lepidixodes*) from bats in Malaya, North Borneo, and the Congo (Acarina-Ixodidae). Acarologia 3(3): 285-290. 1961.

Philip, C. B.: Additional records of Tabanidae from the West Coast of South America (Diptera). Pan-Pacific Ent. 37(2): 111-116. April 1961.

Philip, C. B.: Arthropod vectors in relation to the reservoir mechanism of microbial agents of animal diseases. Acta Tropica 18(3): 257-262. 1961.

Yunker, C. E.: A sampling technique for intranasal chiggers (Trombiculidae). J. Parasitol. 47(5): 720. Oct. 1961.

Tobie, J. E., Burgdorfer, W., and Larson, C. L.: Frozen sections of arthropods for histological studies and fluorescent antibody investigations. Exp. Parasitol. 11(1): 50-55. Feb. 1961.

Philip, C. B.: Three new Tabanine flies (Tabanidae, Diptera) from the Orient. Indian J. Ent. 21(2): 82-88. 1959. (Not received until 1961).

Philip, C. B. and Mackerras, I. M.: On Asiatic and related Chrysopinae (Diptera: Tabanidae). Philippine J. of Sci. 88(3): 279-324. Sept. 1959. (Not received until 1961).

Kohls, G. M. and Hoogstraal, H.: Observations on the subgenus Argas (Ixodoidea, Argasidae, Argas). 4. A. neghmei, new species, from poultry houses and human habitations in northern Chile. Ann. Ent. Soc. Amer. 54(6): 844-851. Nov. 1961.

Kohls, G. M., Hoogstraal, H., and Clifford, C. M.: Observations on the subgenus Argas (Ixodoidea, Argasidae, Argas). 5. Study of A. brevipes Banks, 1908, from birds in Arizona and California, U.S.A., and Baja California, Mexico. Ann. Ent. Soc. Amer. 54(6): 869-877. Nov. 1961.

Clifford, C. M. and Kohls, G. M.: A new distribution and host record for Ixodes muris Bishopp and Smith, 1937 (Acarina:Ixodidae). Proc. Ent. Soc. Wash. 63(3): 210. Sept. 1961.

In press:

Philip, C. B.: Helminths as carriers of microbial disease agents of man and animals. In "Biological Transmission of Disease Agents". Academic Press.

Sonenshine, D. E., Clifford, C. M., and Kohls, G. M.: The identification of larvae of the genus Argas (Acarina: Argasidae). Acarologia.

Kohls, G. M.: Rediscovery of Haemaphysalis mjobergi Warburton, 1926 (Acarina: Ixodidae). Pacific Insects.

Kohls, G. M.: New Guinea trombiculid mites in relation to scrub typhus. Medical Science Publication No. (Symposium on Scrub Typhus) March 3, 1961. AFEB, Walter Reed Army Med. Center.

Kohls, G. M.: A new species of Ixodes from Malaya (Acarina-Ixodidae). Proc. Ent. Soc. Wash.

Philip, C. B. and Kohls, G. M.: Proposal to validate under the plenary powers the specific name Dermacentor andersoni. Internat. Bull. Zool. Nomenclature.

Philip, C. B.: Notes on Palaearctic Nemorius (Diptera: Tabanidae) with description of two new species. Bull. et Annales de la Soc. Roy. d'ent. de Belgique.

Philip, C. B.: Further notes on Far Eastern Tabanidae with description of five new species. Pacific Insects.

Philip, C. B.: Family Tabanidae. In "Catalogue of Nearctic Diptera," Dr. Allen Stone, et al., editors.

Philip, C. B. and Steffan, W. A.: New North American Tabanidae. XIV. An undescribed Apatolestes from the California coast. Pan-Pacific Ent.

Yunker, C. E. and Jones, E. K.: Endoparasitic chiggers: I. Chiroptera, a new host-order for intranasal chiggers, with descriptions of two new genera and species (Acarina: Trombiculidae). J. Parasitol.

Honors and Awards relating to this project:

Dr. C. B. Philip

Elected to Council of American Society of Tropical Medicine and Hygiene.

Requested to give four lectures on tick-borne diseases for Academic Year Institute, University of Georgia, and for two biological fraternities as well as classes in Advanced Medical Entomology.

Provided a half-day lecture on ticks and disease transmission for training course at CDC, Atlanta, Ga.

Invited to give review of work of RML before International Conference of Great Plains Entomologists, Bozeman, and Montana Ass'n. of Managers of Chambers of Commerce, Hamilton.

On thesis committee, University of Utah Dept. of Ent., to assist Capt. Wallace Murdock with his thesis on Tabanidae of Japan and Korea.

Dr. W. Burgdorfer

Invited to become member of N. Y. Academy of Sciences.

Nominated to membership in the American Academy of Microbiology.

G. M. Kohls

Selected as Guest Scholar by Kansas State University, Manhattan. This will involve a 3-day visit there in the spring of 1962 to give lectures and for consultation with zoology-entomology students.

As assistant editor of Journal of Parasitology, reviewed 25 manuscripts submitted for publication in that journal.

Courtesy appointment as Lecturer in University of Montana Department of Zoology to act as major advisor to Mackram Kaiser, an Egyptian student from NAMRU-3, whose thesis will be on ticks.

Dr. J. M. Brennan

Invited to present results of mite and tick studies in Panama before the International Microbiological Congress in December to be held in San Juan, Costa Rica.

Serial No. NIAID-175
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Mechanisms of hypersensitivity and its role in disease

Principal Investigator: S. B. Salvin

Other Investigators: J. E. Coe, M. B. Gregg, J. J. Munoz, J. Nishio, and R. F. Smith

Cooperating Units: None

Man Years (calendar year 1961):
Total: 16.25
Professional: 5.25
Other: 11.0

Project Description:

Objectives:

(1) To establish the mechanism of delayed hypersensitivity, (2) to investigate the relationship of delayed hypersensitivity to other phases of the antigen-antibody reaction, such as tolerance, antibody synthesis, anaphylaxis, or contact allergy, (3) to compare and correlate the biochemical and pharmacologic bases of the various types of hypersensitivity, (4) to determine the role of hypersensitivity in infectious and "auto-immune" diseases.

Methods:

The antigens used are primarily highly purified proteins, although in studies of such auto-allergic diseases as experimental allergic encephalomyelitis and thyroiditis, tissue fractions have been used as antigens. Many immunological and immunochemical procedures are used such as passive cutaneous anaphylaxis, diphtheria toxin-antitoxin intracutaneous rabbit tests, intracorneal tests, cellulose-column chromatography, curtain electrophoresis, immune electrophoresis, induction of auto-immune diseases, tracing of radioactive labeled antigens, quantitative complement determination, and diazotization for production of synthetic protein conjugates. Attempts to use the Schultz-Dale technique will be made.

Part B included: Yes

Serial No. NIAID-175
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Mechanisms of hypersensitivity and its role in disease

Principal Investigator: S. B. Salvin

Other Investigators: J. E. Coe, M. B. Gregg, J. J. Munoz, J. Nishio, and R. F. Smith

Cooperating Units: None

Man Years (calendar year 1961):
Total: 16.25
Professional: 5.25
Other: 11.0

Project Description:

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Part B included: Yes

A specialized procedure for study of tolerance in unborn guinea pigs has involved "in utero" injection of the fetus. Pregnant guinea pigs are subjected to laparotomy, at which time the uterus is exteriorized and the fetus injected intraperitoneally with purified protein as antigen. The intact uterus is replaced and the incision closed. After birth, the offspring are skin tested and sensitized with various antigens to determine the degree of tolerance and hypersensitivity.

Major findings:

Three methods have been described from this laboratory for the production of delayed hypersensitivity with purified proteins: (1) inoculation of antigen-antibody precipitates in antibody excess, (2) intradermal introduction of minute amounts of antigen and, (3) introduction of small quantities of hapten-protein conjugates, wherein antibody is produced to the hapten and delayed hypersensitivity to the protein.

1. Contact hypersensitivity.—Contact hypersensitivity, which is analogous to the delayed type, is induced either by frequent application of a simple chemical on the skin or by intradermal injection of this chemical in a water-in-oil emulsion. Injection of a conjugate containing the hapten and a purified protein results in antibody to the hapten but does not induce contact hypersensitivity to the hapten. When a conjugate of such a simple chemical with a soluble fraction from guinea pig skin is used as sensitizing antigen, contact hypersensitivity to the simple chemical results, as well as delayed hypersensitivity to the protein and circulating antibody to the simple chemical. These observations are consistent with the belief that the specificity of contact hypersensitivity is directed toward a particular skin protein which has been altered by combination with the simple chemical and as a corollary that sensitization with a conjugate of hapten and a heterologous protein does not result in contact hypersensitivity.

2. Influence of antibody on delayed hypersensitivity.—Since antibody formation seems associated with the development of delayed hypersensitivity and since delayed hypersensitivity may be induced by injection of antigen-antibody precipitates in antibody excess, the influence of antibody on delayed hypersensitivity and on further formation of antibody was investigated. For this purpose, conjugates of a hapten with a purified protein were used. When delayed hypersensitivity to a protein was induced in the absence of circulating antibody and the animal subsequently injected with a conjugate of homologous protein and a new hapten, an anamnestic response to the hapten occurred. When, however, antibodies to the protein portion of this conjugate were present, the anamnestic response to the hapten was inhibited. These results reflect the basic difference in specificity between delayed hypersensitivity and antibody formation wherein the specificity of the delayed phase is controlled by broad portions of the protein molecule and the antibody phase is directed toward more limited sections of the antigen molecule. Since serum antibody seems to inhibit additional

antibody formation but delayed hypersensitivity enhances antibody formation, experiments are in progress to determine further the relationship between the two. The introduction of antibody before, during, or after a period of delayed hypersensitivity suppresses immediately further antibody formation. The significance of this suppression in the immune process is being investigated.

3. Thyroid and delayed hypersensitivity.—The influence of hormones on hypersensitivity and antibody production is being investigated. For example, cortisone suppresses the antibody-forming process, but has little if any influence on delayed hypersensitivity. Thyroxin, on the other hand, enhances the antibody response, but has little effect on delayed hypersensitivity. Thyroxin also had no detectable influence on the induction period between the time of antigen injection and the appearance of detectable quantities of circulating antibody. Experimental allergic encephalomyelitis (EAE), however, which is considered a manifestation of delayed hypersensitivity, was definitely inhibited in hyperthyroid guinea pigs. The onset of disease tended to occur later and was of decreased severity in the thyroxin-treated animals versus the controls. In an effort to understand this difference in susceptibility to EAE, other parameters were measured in order that the mechanism whereby EAE is suppressed in thyrotoxic animals can be elucidated further. Because pyridoxine affects antibody production and because changes in the physiology of vitamins have been associated with hyperthyroidism, the effect of pyridoxine on delayed hypersensitivity is being studied.

4. Corneal reactions.—Measurement of delayed hypersensitivity has been difficult because quantitative *in vitro* tests do not exist and because the diameter of induration of the skin lesion of sensitized animals does not reflect the degree of hypersensitivity. Responses following injection of specific antigen into the cornea of a hypersensitive animal are believed to be due solely to delayed hypersensitivity since the cornea is not vascularized. When a conjugate of hapten and protein is used as antigen, delayed hypersensitivity appears to the protein and circulating antibody to the hapten. In the cornea, however, reactions occur only to the protein moiety of the conjugate, although circulating antibody and Arthus reactions may be present in other tissues of the guinea pig. The cornea does not respond in a manner associated with circulating antibody. Passive transfer of sensitized cells into a normal recipient produces a delayed hypersensitivity which can be detected by intracorneal testing. The corneal response is primarily polymorphonuclear in type. Further experiments indicated that leucocytes from nearby tissues and blood vessels responded chemotactically to antigen in the cornea. The cells of the cornea, therefore, are merely the site of the allergic response and play little or no role in actually eliciting it.

5. Delayed shock.—Delayed or tuberculin shock supposedly is a systemic manifestation of delayed hypersensitivity and not related to

the presence of circulating antibody but to the presence of sensitized cells. From experiments in guinea pigs with hapten-protein conjugates, it has been suggested that delayed shock is a combined reaction, with its characteristics dependent on both delayed hypersensitivity and circulating antibody. For example, when only delayed hypersensitivity to a conjugate is present, intraperitoneal injection of the protein produces a hyperthermia characteristic of the systemic delayed reaction. Intraperitoneal injection of the hapten does not produce detectable systemic changes. Later, however, when delayed hypersensitivity is present to the protein moiety and circulating antibody appears with its specificity directed toward the hapten, intraperitoneal injection of the conjugate produces a brief initial hyperthermia followed by a distinct hypothermia, a lymphopenia, and occasionally may cause death. Injection of the protein carrier alone produces only a hyperthermia with no other manifestation of systemic shock. Injection of the hapten attached to a heterologous protein does not produce symptoms characteristic of delayed hypersensitivity, such as hyperthermia, but produces signs typical of protracted anaphylactic shock, namely hypothermia, lymphopenia, and occasionally death in 2 to 4 hours. Further emphasis on the dual nature of delayed systemic shock is provided by experiments on desensitization, the influence of antihistamines, and the effect of cortisone.

6. Hypersensitivity in the newborn.—The immune processes of newborn guinea pigs are different from those of adults. When animals are sensitized within 12 hours after birth a delayed type of response to intradermal injection can not be evoked for about 14 days after birth. During this period of resistance to intradermal responses to protein, other processes associated with delayed type of hypersensitivity can be induced. For example, many of the animals will respond to sensitization and subsequent skin testing with contact haptens. Also, newborn animals respond similarly to adults in the expression of EAE and will eliminate radioactive antigen at the same rate as adults. When quantities of antigen sufficient to induce delayed hypersensitivity in adults are injected into newborns and are followed by a second injection of the same specific antigen, an anamnestic response follows similar to that in adults. These data suggest, therefore, that delayed hypersensitivity does exist in newborn animals but that the state of hypersensitivity in the skin is not expressed. This belief is further enhanced after studies on "passive transfer" of sensitized cells. When cells from the lymphnodes of sensitized newborn guinea pigs were injected intraperitoneally into normal adult recipients and when such adults were skin tested, a typical delayed response has been observed.

The belief is entertained that the inflammatory response in the newborn is defective. More extensive knowledge of the exact nature of this unresponsiveness of the skin may provide methods for restricting the response in adults.

7. Quantitative "in vitro" determination of delayed hypersensitivity.—Early attempts in this laboratory to measure the degree of delayed hypersensitivity with tritiated protein were unsuccessful. Subsequently, proteins such as bovine gamma globulin (BGG) or hen egg albumin (HEA) were labeled with the radioactive I^{131} and injected intravenously or intradermally into normal guinea pigs, guinea pigs with delayed hypersensitivity but without circulating antibody, and guinea pigs with circulating antibody. Although useful information was obtained, features of antigen elimination were not detected which could be used as a quantitative index of delayed hypersensitivity. A method has now been devised whereby the amount of hypersensitivity in cells from hypersensitive donors can be determined in vitro. Studies are in progress to perfect this technique, and thereby make possible studies on the influence of different amounts of delayed hypersensitivity on various related immune responses.

8. Delayed hypersensitivity and tolerance.—Since guinea pigs show delayed hypersensitivity readily, experiments on tolerance were attempted with this species of animal. This phenomenon has been demonstrated in adult animals by feeding contact haptens in large and extended doses. The optimum conditions for inducing tolerance to simple chemicals in guinea pigs are being investigated. The tolerance has been shown to be specific in that it applies specifically to suppression of contact hypersensitivity and to antibodies against the simple chemical. The tolerance is usually not complete, since 10 to 20% of the animals may have a faint reaction upon initial challenge with the contact hapten. The tolerance can, however, be reduced or abolished by continued sensitization with the hapten or by utilization of mycobacterium with Freund's adjuvant in the sensitization procedure. Also, antibodies to the hapten are produced at normal times in tolerant animals when these animals are sensitized with a conjugate of specific hapten and a carrier protein. The specificity of the tolerance is also being investigated with the aid of such haptens as dinitroflurobenzine (DFB), and picryl chloride (PiCl). Antibodies to a hapten such as DFB were formed in tolerant animals when they were sensitized with the conjugate, DFB-guinea pig serum. However, these antibodies were present in lower titer and for a shorter duration in the tolerant animals than in the controls. In tolerant animals sensitized with DFB-HEA and DFB-BGG, the time of appearance and the specificity of the antibodies were the same as in the controls. Antibodies to the carrier protein HEA and BGG were not found in either group.

Tolerance has also been induced in guinea pigs by injection of the fetus with large quantities of a specific protein. After birth the resulting guinea pig may be unable to develop hypersensitivity to the antigen to which the animal had been exposed in utero. Some of these "tolerant" animals eventually do form antibodies and do develop responses to intradermal injection of antigen. Others, however, over a period of several months after birth, did not show responses or circulating antibodies after specific sensitization.

9. An antigen has been prepared by physicochemical procedures from the pathogenic fungus, Cryptococcus neoformans and its biochemical properties determined. The active fraction produces extensive induration in the skin of guinea pigs which previously had been infected experimentally with C. neoformans. This antigen is now being tested in several large cities in order to determine whether cryptococcosis occurs as a mild disease presumably associated with a pulmonary infection. The preparation of this specific skin testing antigen thus offers a new means for detection of the prevalence of the disease in man.

10. At the present time, extracellular pharmocologic substances have not been shown to be associated with the process of delayed hypersensitivity. Histamine is present in lymphocytes which migrate toward the area of intradermal testing, although this and similar substances have not been associated with the process of hypersensitivity. Experiments are underway to learn whether such compounds as histamine, complement, and the like have any role in delayed hypersensitivity.

11. If delayed hypersensitivity is associated with antibody formation, then the possibility presents itself that gamma globulin may form the basis for delayed hypersensitivity either in an intracellular form or in extracellular form. Experiments on this particular problem are in progress.

12. Fractions of gamma globulin have been prepared and their role in hypersensitivity is being investigated.

Significance:

Allergic diseases are extremely common and of great importance in man. In recent years, many diseases for which an etiologic agent has not been known have been shown to be due to immunologic disturbances rather than infectious processes. These diseases are the result of the development of hypersensitivity to autologous substances in the body. In addition, the rejection of tissues has been indicated to be associated with the basic processes of delayed hypersensitivity. Not only homograft rejection but also tumor rejection are included in this category. In addition, an important part of pathophysiology due to chronic infectious diseases, such as tuberculosis and histoplasmosis, is believed to be associated with delayed hypersensitivity rather than specific circulating antibodies to the microorganism. Although it is increasingly evident that delayed hypersensitivity is most important in certain diseases of man, the mechanism of delayed hypersensitivity and its relationship with other immune processes are not understood. If the mechanism of delayed hypersensitivity is clarified, then advances presumably could be made in the protection of man and animals against conditions or diseases associated with hypersensitivity.

Proposed course:

Investigations on the mechanism of delayed hypersensitivity and its association with other immune phenomena will be continued. Attempts will be made to perfect the *in vitro* method of quantitative determination of delayed hypersensitivity. Also, with the aid of inbred animals, both guinea pigs and mice, efforts will be continued to determine what type of "antibody" is associated with the hypersensitivity process. Collaboration with Dr. Ribi, Dr. Munoz, and Dr. Malmgren is planned in the study of the purification of the protective antigen of *B. pertussis*. Efforts also will be continued (Dr. Munoz) toward the isolation from *B. pertussis* cells of the histamine sensitizing factor and the factor that increases anaphylactic sensitivity in mice.

Serial No. NIAID-175

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Salvin, S. B. and Smith, R. F.: The specificity of allergic reactions. III. Contact hypersensitivity. J. Exp. Med. 114(2): 185-194. Aug. 1961.

Salvin, S. B. and Gregg, M. B.: The specificity of allergic reactions. IV. The cornea. Proc. Soc. Exp. Biol. & Med. 107(3): 478-483. July 1961.

In press:

Salvin, S. B. and Smith, R. F.: An antigen for detection of hypersensitivity to *Cryptococcus neoformans*. Proc. Soc. Exp. Biol. & Med.

Salvin, S. B.: Immunologic aspects of the mycoses. Eleven chapters on: actinomycosis, aspergillosis, candidiasis (moniliasis), chromoblastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis, nocardiosis, North American blastomycosis, sporotrichosis, dermatophytosis. Progress in Allergy, Paul Kallós, ed., Basel, S. Karger.

Honors and Awards relating to this project:

Dr. S. B. Salvin

Guest speaker at IV International Congress of Allergology, New York City, October, 1961.

Lecturer in Immunology at Montana State University, Missoula.

Invited to participate as a lecturer in the high school science program sponsored by the Montana Academy of Sciences.

Dr. J. J. Munoz

Associate Editor, Journal of Immunology, 1961.

Special Lecturer at University of Washington Medical School, Seattle, Washington, June 20 - August 20, 1961.

Serial No. NIAID-176
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Viruses, virus components, and virus surfaces

Principal Investigator: B. H. Hoyer

Other Investigators: J. A. Duerre, R. K. Gerloff, R. A. Ormsbee, and D. B. Ritter

Cooperating Units: University of Kansas (Dr. Jacob Duerksen) and University of Washington (Drs. John Holland and Milton Gordon)

Man Years (calendar year 1961):
Total: 11.5
Professional: 3.5
Other: 8.0

Project Description:

Objectives:

Success of the radioisotope precipitation (RIP) test as applied to radio-labeled poliovirus particles and *Coxiella burnetii* cells has prompted current emphasis of this test, now unique to the Rocky Mountain Laboratory, to study problems related to virus antigen and antibody. The application of the RIP test to problems concerning the appearance of specific antigens when animal cells are exposed to plant virus and bacterial nucleic acids is in progress. The RIP test has also been applied to field investigations of Q fever reported elsewhere.

Methods:

Either highly purified viruses, rickettsiae, or other antigens are presently required; these materials with a radiolabel which is present only in the antigen of interest, even in an impure system, are also satisfactory. Specifically labeled poliovirus is prepared by infecting tissue culture cells with poliovirus in the presence of P^{32}-orthophosphate and purifying the resulting labeled virus by chromatography on DEAE cellulose as previously described. Purified, ether inactivated *C. burnetii* cells are made radioactive by iodinating their protein constituents with I^{131}.

Part B included: Yes

The RIP test can be outlined as follows: A^* = radiolabeled antigen; A = nonlabeled antigen; Ab = antibody versus the antigen; AGG = antibody (antiglobulin) prepared against globulin of the type which contains the antibody.

To detect Ab:

$A^* + Ab$; incubate 37°C for 1 hour.

$(A^* + Ab) + AGG$; incubate 37°C for 1 hour.

If specific Ab is present it combines with A^* and this complex subsequently reacts with AGG. Mild centrifugation removes the $(A^* + Ab + AGG)$ complex from the supernatant fluid. The percent radioactivity which disappears from suspension is determined by radioisotope detection procedures and provides a quantitative measure of Ab content of a series of dilutions of antisera.

To detect A (inhibition type test):

A + limiting Ab; incubate 37°C for 1 hour.

$(A + Ab) + Ab + A^*$; incubate 37°C for 1 hour.

$(A + Ab) + (A^* + Ab) + AGG$; incubate 37°C for 1 hour.

Mild centrifugation removes from suspension the A^* which combined with Ab still free after reaction with A. If all Ab were combined with A no A^* would be precipitated. Gradations of combination of A^* and Ab are detected by radioisotope counting devices.

Major findings:

1. With poliovirus and C. burnetii cells as models of antigens with a size spectrum of 25 mμ to 300 mμ the RIP test provides a sensitive means of detecting both bivalent and monovalent antibody. No removal of excess globulin is necessary as it is in conventional Coombs type tests where visible precipitate, rather than removal of radioactivity from suspension, is the indicator. In the poliovirus system 0.1 to 0.001 μg of antigen can be detected by the inhibition type test. The results for both antigen and antibody detection are quantitative and, if necessary, can be obtained in 2-3 hours. The sensitivity of detection of poliovirus antibody is routinely 30 X that of the tissue culture neutralization test and for C. burnetii antibody 100 X that of the capillary agglutination and complement fixation tests. Inclusion of antiglobulin is essential for this degree of sensitivity and both the small poliovirus particles and the large rickettsial particles are satisfactory antigens which react specifically with their antibody.

2. Cells infected with poliovirus produce antigen detectable with the RIP inhibition test at the same time and at the same rate that whole virus is formed. This substantiates results obtained by Darnell and associates with other procedures and makes it extremely unlikely that antigen other than that combined with whole virus is produced as a result of poliovirus infection.

3. When radiolabeled poliovirus is combined with antibody and large multiplicities of unlabeled virus to labeled virus are added there is little reversal (release of radio-labeled virus from antibody) as detected by decreased precipitation of radio-labeled virus when anti-globulin is added. This is probably due to the large number of antibody combining sites on the poliovirus surface and the relatively large size of the poliovirus particle.

4. A hyperimmune monkey serum will neutralize poliovirus when about 45×10^4 antibody molecules are present per particle in suspension and at the endpoint of the RIP reaction 50-150 molecules of antibody per particle are present.

5. When poliovirus is irradiated with ultraviolet light it can be inactivated and still be a satisfactory RIP antigen. Irradiation beyond limits where active virus can be detected results in loss of P^{32} from labeled virus RNA; the radioactivity is no longer sedimentable under conditions which sediment whole virus. Irradiation therefore demonstrably alters virus structure.

6. Poliovirus antibody can be detected in some individuals vaccinated with Salk vaccine who had no antibody detectable by tissue culture neutralization tests. The RIP titers in individuals with detectable neutralizing antibody were proportional to the serum neutralizing titers but much higher.

7. When active virus is used in the RIP test, disappearance from suspension of radioactivity and active virus (as determined by PFU) are parallel over a series of specific antibody dilutions. About 10^5 PFU are required in this test so that radioactivity is sufficient for convenient measurement. If the antigen is diluted so that 10^2 PFU are present the disappearance of PFU from suspension again parallels that of radioactivity in the system containing 10^5 PFU; antibody titers obtained with the diluted virus are somewhat higher than those with 10^5 PFU. A modification of the RIP test using nonlabeled virus is, thus, possible.

8. C. burnetii cells are good RIP antigens when iodinated with I^{131}. Poliovirus which is highly purified sometimes is a good antigen when radioiodinated. Because specific activities of 1-2 mc/mg protein can be obtained by radioiodination, the I^{131}-label is very desirable. Systems for consistently satisfactory radioiodination of poliovirus are under study.

9. Since the RIP test requires that antibodies combine with a measurable portion of antigen, decreasing the number of antigen particles or increasing the size of the antigen particle so that sufficient radioactivity is present in a small number of particles is desirable. It also would be desirable to use an impure, nonradio-labeled antigen combined with a large radio-labeled particle. A successful lead in this direction has been obtained with bentonite particles. Iodinated bovine gamma globulin was combined with bentonite in the presence of nonlabeled poliovirus. The resulting complex served as a satisfactory antigen in the RIP test. If this procedure can be standardized the RIP test will be available for any nonlabeled antigen which can combine with a particle such as bentonite. Latex particles have been extensively tried but are not satisfactory as a base to hold RIP antigens.

10. Dr. John Duerre has recently joined our group. His interest in nucleosidases will be applied to tissue culture cells both normal and virus-infected. In the short time that he has been in the laboratory he has successfully accomplished 30- to 50-fold purification of nucleosidases so that the properties of these enzymes may be determined.

Significance:

The RIP test is adaptable to the sensitive, rapid, and quantitative detection of antibody and antigen. It overcomes the difficulties of quantitation inherent in the widely applied fluorescent antibody technique and will serve to augment this procedure. It can be used as a very sensitive screen test for antibody and should be of great value to virus epidemiology. It is also a quantitative test which has been applied to fundamental problems (i.e., appearance of poliovirus protein in infected cells) and should be widely applied in other laboratories in the future. If the test in which bentonite particles are used can be adapted to a wide variety of antigens, it should be of importance in the tumor virus field.

Proposed course:

Further applications of the RIP test to fundamental problems of viruses and viral antigens will be explored. In particular, particles to carry nonlabeled antigens (i.e., the bentonite system) will be further investigated. The specificity of the RIP test will be exploited in the psittacosis virus group where nonspecificity of present techniques is a problem. We intend to continue contact and cooperative investigation with Dr. Jacob Duerksen, University of Kansas, on transfer of information from RNA of bacteria to animal cells and Drs. John Holland and Milton Gordon, University of Washington, on transfer of information from tobacco mosaic virus RNA to animal cells. The RIP test and enzymologic techniques will be used to detect such antigens and enzymes as these RNAs may stimulate. Column chromatography and biophysical techniques will continue to be used to investigate properties of viruses before

and after interaction with cells. As new procedures become available or are developed here they will be applied to these problems. It is anticipated that Dr. Duerre will supply a new field of interest through his knowledge of enzymologic approaches, particularly those concerned with nucleosidases.

Serial No. NIAID-176

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Honors and Awards relating to this project:

Dr. B. H. Hoyer

Chairman 4th Annual Meeting of Montana Microbiologists.

Lecturer in microbiology, Montana State University, Missoula.

Resident advisor for Ph.D. thesis of R. K. Gerloff, University of Minnesota.

Invited to present discussions on virus purification and radioisotope precipitation test; St. Louis University, Feb. 1961; CDC, Atlanta, Ga., April 1961.

Invited to participate as a lecturer in the high school science program sponsored by the Montana Academy of Sciences.

R. K. Gerloff

Invited to present discussion on radioisotope precipitation test, University of Minnesota, May 1961.

Dr. R. A. Ormsbee

Invited to participate as a lecturer in the high school science program sponsored by the Montana Academy of Sciences.

Serial No. NIAID-177
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Immune prophylaxis of mycobacterial infections

Principal Investigator: E. Ribi

Other Investigators: W. T. Haskins, C. L. Larson, and W. C. Wicht

Cooperating Units: None

Man Years (calendar year 1961):

Total:	6.75
Professional:	1.75
Other:	5.0

Project Description:

Objectives:

The objectives of this program are: (a) to develop methods for assay of living vaccines (i.e., BCG) for prevention of tuberculosis, (b) to develop other types of vaccines useful in prophylaxis of this disease, and (c) to isolate fractions of tubercle bacilli by chemical and physical methods and to study the biological activity of such fractions.

Methods:

The methods which have lent distinction to the program have been the use of a refrigerated cell fractionator to disrupt tubercle bacilli and the use of aerosols to administer virulent and avirulent organisms to experimental animals.

Major findings:

1. The AED_{50} (median effective allergic dose) of cell walls from Mycobacterium butyricum or M. tuberculosis required to act as an effective adjuvant in producing isoallergic encephalitis in guinea pigs was 0.1 mg when the antigenic mixture was injected into the nuchal area and was about 10-fold less when injected into the foot pad. A significant finding was the observation that when cell-wall material was solublized

Part B included: Yes

the addition of oil or an emulsifying agent was not necessary to produce an adjuvant effect of the same order.

2. Resistance induced by mixtures of live and heat-killed attenuated tubercle bacilli (H37Ra) appears to be proportional only to the number of viable organisms present.

3. Guinea pigs and rabbits inoculated intradermally with phenol-extracted bacilli do not develop local lesions and do not develop delayed sensitivity. They develop circulating antibodies. These results are obtained only with young cultures of BCG. Preliminary experiments suggest that vaccines prepared by phenol extraction of 4-day-old cultures of BCG are capable of producing resistance in mice.

Significance:

The most important finding is related to the use of phenol-extracted young cultures (4 days old) of BCG. The fact that young organisms, when subjected to 2% phenol for 90 hours, yield a product which is nontoxic, nonallergic and, hopefully, immunogenic suggests that it may be possible to develop a nonliving vaccine which may be effective in producing resistance against tuberculosis. Since only young organisms yield such a product, it is thought that the cell wall of these bacilli must differ in respect to the firmness with which fatty acids and lipids are incorporated into them. If an effective vaccine which is nontoxic and nonallergic can be produced, many of the problems relating to immune prophylaxis of tuberculosis would be solved.

Proposed course:

Studies will be extended to determine the effect of phenol-extracted tubercle bacilli upon the course of tuberculosis in mice. Systematic studies of the role of aging on the ability to delete toxic and allergenic compounds will be pursued, and the reason for the changes resulting from age will be sought through electron microscopic studies of sections of tubercle bacilli of varying ages, and through chemical analysis of the cell walls prepared from them.

Serial No. NIAID-177

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Larson, C. L., Ribi, E., Wicht, W. C., and List, R.: Skin reactions produced in rabbits by cell walls and protoplasm of *Mycobacterium tuberculosis* and *M. butyricum*. Am. Rev. Resp. Dis. 83(2) Pt. I.: 184-193. Feb. 1961.

Serial No. NIAID-178
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Immune prophylaxis of Salmonella infections

Principal Investigator: E. Ribi

Other Investigators: R. L. Anacker, W. T. Haskins, M. T. McKee, K. C. Milner, and D. B. Ritter

Cooperating Units: NCI (Drs. Maurice Landy and Willie Smith), University of Florida (Dr. Emanuel Suter), and University of Buffalo (Dr. Erwin Neter)

Man Years (calendar year 1961):

Total:	8.0
Professional:	3.0
Other:	5.0

Project Description:

Objectives:

The features of composition and structure of bacterial endotoxins required for expression of characteristic host reactivity are investigated.

Methods:

Biophysical, chemical, and immunological approaches are combined in this project. The methods used for isolation and refinement of endotoxins have been, in a large measure, developed at the Rocky Mountain Laboratory.

Major findings:

Previous efforts to strip off extraneous material, such as protein and lipid, in which some investigators believe the ultimate toxic principle resides, have yielded predominantly polysaccharide products containing only small amounts of fatty acids and nitrogenous material. These refined products, which defy exact structural analysis, are still large complexes whose chemical composition closely resembles haptenic polysaccharide obtained by acid hydrolysis of endotoxin. Inasmuch as

Part B included: Yes

biological activity was associated with large complexes, while the much smaller units derived from them were inactive, it was visualized that host reactivity could be dependent upon macromolecular properties.

This hypothesis was tested by determining changes in physicochemical and biological properties in the endotoxin complex during hydrolysis with dilute acetic acid. To this end, products of hydrolysis removed at various intervals were examined in the optical ultracentrifuge and were also assayed for pyrogenicity, tumor-damage, lethality, and protection. Findings obtained with these parameters were consistently reproducible and revealed that the rate at which the host-reactive properties were abolished paralleled the rate of dissociation of the complex into haptenic units whose size was of the order of 1/100 that of the original endotoxin. Thus, the ultracentrifugation patterns clearly show that, as the area under the fast-moving endotoxin boundary was reduced during progressive hydrolysis, the area under the slow-moving haptene boundary increased correspondingly. The depolymerization could then be quantitated by determination of the sedimentation constants and by measuring the areas under the "Schlieren" boundaries. The potency remaining at various stages of hydrolysis could be accounted for by remnants of undissociated endotoxin. For example, in the neutralized product of endotoxin which still displayed appreciable activity after exposure to hydrolysis for 15 minutes, the ultracentrifuge revealed the presence of the slow- and fast-moving components in about equal quantity. The two components, which also are discernible in agar gel diffusion, were separated by subsequent differential centrifugation. The slow-moving component was found to be essentially inactive whereas the fast-moving component possessed the same high biologic potency as the zero time sample.

During a search for a method to circumvent problems associated with the well-known low solubility of endotoxins in water, it was found that after refined endotoxins were exposed to hydrogenolysis with metal hydrides, highly water soluble aluminum citrate complexes were formed which completely retained host-reactive potency (instant endotoxin).

A simple, rapid method for removing protein from endotoxin complexes was developed to replace conventional tedious and time-consuming procedures. A single treatment allows reduction of the nitrogen content from about 6% to about 0.5%. About one-half of the remaining nitrogen can be accounted for by the amino sugars present. This represents the lowest nitrogen so far reported for endotoxins.

Significance:

These findings seem to provide an explanation of the major requirement for endotoxin to elicit host reactions, i.e., a macromolecular complex of critical size. It would, however, be premature to conclude that the effect of acid is merely one of splitting of bonds by which

particles of the size of a haptene are held together, inasmuch as other less apparent changes could also be a consequence of such treatment.

Proposed studies:

Experiments are planned to determine whether other agents which destroy biological activity of endotoxins, such as alkali, periodate, and host enzymes also bring about depolymerization or whether they produce still other alterations in these complexes. In addition, a search will be made for agents which may cause depolymerization with retention of biological activity and for agents which may recombine haptenic particles and reestablish endotoxic potency.

Serial No. NIAID-178

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Haskins, W. T.: Spectrophotometric determination of fatty acid amides in lipides. Analytical Chemistry 33(10): 1445-1446. Sept. 1961.

Ribi, E., Haskins, W. T., Landy, M., and Milner, K. C.: Preparation and host-reactive properties of endotoxin with low content of nitrogen and lipid. J. Exp. Med. 114(5): 647-663. Nov. 1961.

Haskins, W. T., Landy, M., Milner, K. C., and Ribi, E.: Biological properties of parent endotoxins and lipoid fractions, with a kinetic study of acid-hydrolyzed endotoxin. J. Exp. Med. 114(5): 665-684. Nov. 1961.

In press:

Ribi, E., Haskins, W. T., Landy, M., and Milner, K. C.: Symposium on bacterial endotoxins. I. Relationship of chemical composition to biological activity. Bact. Reviews (Dec. 1961).

Honors and Awards relating to this project:

Dr. E. Ribi

Invited to participate as a chairman in a conference on endotoxins at Bryn Mawr College, October 1961.

Invited to lecture in microbial biophysics at Bryn Mawr College, Pennsylvania, in May 1962.

Invited to give a seminar on endotoxins at the National Institutes of Health, Bethesda, Md., May 1961.

Serial No. NIAID-179
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: The role of morphological elements of microorganisms in immunity and related phenomena

Principal Investigator: E. Ribi

Other Investigators: R. L. Anacker, C. L. Larson, and K. C. Milner

Cooperating Units:
Ivan Sorvall, Inc., Norwalk, Connecticut;
Parke, Davis and Co., Inc., Detroit, Michigan;
Merck, Sharp and Dohme, West Point, Pennsylvania;
University of Michigan (Dr. Philip Gerhardt);
University of West Virginia (Mrs. Gwen Stewart)

Man Years (calendar year 1961):

Total:	5.0
Professional:	1.0
Other:	4.0

Project Description:

Objectives:

The activities of this section include collaborative studies with investigators from other institutes who are interested in applying our biophysical approaches to their specific problems in investigations on a variety of microorganisms. This year the work has been primarily directed toward perfecting a practical self-contained refrigerated cell fractionator suitable for commercial distribution and specially designed to rupture microorganisms and cells of mammalian and plant tissues without significantly altering their components (cell walls or membranes and cytoplasmic components including microsomal particles, enzymes, and nucleic acids).

Methods:

Through cooperation with designers and engineers from a firm specializing in the manufacture of laboratory instruments, components and prototypes of the cell fractionator were developed and tested. So far, the principle found to be most effective is based upon the release

Part B included: Yes

of pressurized biological material at a refrigerated orifice of a needle valve. For example, when a microbial suspension is processed, as the cells emerge through the orifice the membranes are cracked and protoplasm is released in a dispersed state. The two main morphological elements may then be separated by centrifugation procedures.

Experiments were conducted to determine the extent to which this new instrument is applicable to bio-medical research. All fractionation procedures were checked and controlled by electron microscopy.

Major findings:

The new refrigerated cell fractionator produced for commercial distribution is based on experimental models developed at the Rocky Mountain Laboratory. It is a self-contained unit consisting of a refrigerated high pressure cell (cylinder and piston) and decompression valve, a fill valve which permits filling during down stroke of piston for semi-continuous operation, motor driven hydraulic system capable of maintaining any selected pressure up to 60,000 psi on the 1.5" diameter cell. The orifice temperature can be maintained between 0° and 10°C depending on the flow rate of the effluent. This prevents denaturation of the products and preserves the activity of heat-labile antigens, toxins, enzymes, etc.

Limited amounts of walls and protoplasm from a number of microbial cells have been prepared with the aid of shaking and grinding devices by other investigators, but with this new instrument it is now possible to produce these fractions from a wide variety of microorganisms in quantities adequate for extensive experimentation. Pressures can be selected and regulated according to the rigidity of the walls of the microorganisms to be processed. As evidence of the versatility of the instrument, the following accomplishments are listed:

1. Purified cell-wall and protoplasmic fractions have been prepared from Histoplasma capsulatum, Blastomyces dermatitidis, Cryptococcus neoformans, Candida albicans, Penicillium chrysogenum, Streptococcus pyogenes, Listeria monocytogenes, Serratia marcescens, Escherichia coli, Bordetella pertussis, and several species of Salmonella, Brucella, and Mycobacteria. No evidence of alteration of potency of immunologically active substances (antigens, toxins, allergens) due to exposure to high pressures was observed.

2. Enzymes have been released from fungi and bacteria. Nearly complete breakup was achieved at a concentration of 250 mg wet wt. per ml. Thus, the typical decrease in enzyme activity due to high dilution can be avoided. E. coli disrupted in the cell fractionator had about 20% increase in enzyme activity over cells broken with the Raytheon ultrasonic oscillator. Enzyme activity of protoplasm released from Saccharomyces cerevisiae was comparable to that given by protoplasm

released by disrupting this fungus in the Halvorson colloid mill. By cracking the rigid exosporia and spore coats, a cytoplasmic extract of botulinum spores was prepared under sufficiently anaerobic conditions to maintain dehydrogenase activity. Thus, for the first time, to our knowledge, it was possible to release enzymes from bacterial spores.

3. The fragile membranes of erythrocytes were readily isolated when low pressures were used to rupture the cells. Tissue cultures could be processed to yield completely clear solutions. The same was true when kidney and liver sections were processed after they had been previously homogenized in a Waring blendor. However, attempts to solublize connective tissue have been unsuccessful.

4. In collaboration with Mrs. Gwen Stewart, a guest worker from the University of West Virginia, Clostridium botulinum spores were processed in the cell fractionator. The exosporia were ruptured and could be separated from the spore proper by sedimentation through glycerol gradients. The spore proper, after treatment with EDTA, was then reprocessed to crack the coats. The cytoplasmic constituents were released in a dispersed state and the coats were collected by centrifugation. Thus, the separation of spores into their main morphological elements by this scheme of fractionation enables the study of biological properties, chemical composition, and structure of spores. X-ray diffraction powder diagrams revealed the presence of a crystalline lattice in both exosporia and spore coats.

Significance:

The perfected refrigerated cell fractionator, which is an original design developed at RML, was found to be a valuable new research tool in the area encompassing structure and function of microorganisms. It permits the preparation of purified cell walls from a greater variety of microorganisms than was possible with other devices and also meets the demand for large quantities necessary for extensive experimentation. This is particularly true for studies dealing with isolation and characterization of protective antigens to be used in commercial vaccines.

The isolation of subcellular particles is becoming of increasing importance in biochemistry and biophysics, and efficient methods for controlled disruption of the living cells are therefore in great demand. Since the use of the refrigerated cell fractionator permits the microbial cytoplasm to be released in a dispersed, unaltered state, this instrument can be used for studies on enzymes, microsomal particles, and nucleic acids. The method is also expected to be applicable to basic studies with mammalian and plant tissue cells.

Proposed course:

Collaborative studies and consultative work with other investigators who desire to make use of our methods will be continued.

Based on past inquiries, the applicability of the cell fractionator for isolation of membranes and internal material of larger viruses, mitochondria, nuclei, and chloroplasts will be investigated.

An attempt will be made to construct a micro pressure cell to be used for special purposes at extremely high pressures. With such an instrument it may be possible to remove coats from the cores of small viruses and to dissociate or split macromolecular complexes such as bacterial endotoxins into biologically active and inactive entities.

Serial No. NIAID-179

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

In press:

Perrine, T. D., Ribi, E., Maki, W., Miller, B., and Oertli, E.: A production-model press for the preparation of bacterial cell walls. J. Appl. Microbiol.

Honors and Awards relating to this project:

Dr. E. Ribi

Asked to serve as a member on a "Ph.D." committee for Tulane University Medical School.

Invited to lecture on "Investigations of the role of morphological elements of microorganisms in immunity and related phenomena" at the Institute of Microbiology, Rutgers University, Princeton, N. J.

Serial No. NIAID-180
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: The encephalitides

Principal Investigator: C. M. Eklund

Other Investigators: W. Burgdorfer, R. C. Kennedy, D. B. Lackman, V. F. Newhouse, W. A. Rush, and L. A. Thomas

Cooperating Units: Arctic Health Research Center

Man Years (calendar year 1961):

Total:	15.0
Professional:	3.0
Other:	12.0

Project Description:

Objectives:

Emphasis is placed on the study of the ecology of Western equine (WEE) and St. Louis (SLE) encephalitis viruses since they are the only mosquito-transmitted viruses shown to cause human disease in the western United States.

A member of the California group of viruses from *Aedes trivittatus*, a member of the Buhyamwera group of viruses from *Culiseta inornata*, the Turlock virus, and Anopheles A virus have been isolated. Since human disease has not been attributed to these viruses in the United States, an intensive study of their ecology is not attempted, although incidental ecological information is obtained in connection with the study of the ecology of the WEE and SLE viruses.

Powassan virus is the only representative of the RSSE group of viruses found in the western hemisphere. Nothing is known of its ecology and little about its importance as a cause of human infection. This laboratory is attempting to determine whether the Powassan virus is of any importance as a cause of human infection in the United States and to determine its ecology in this general area.

Part B included: Yes

Methods:

Mosquitoes, ticks, and tissues from vertebrate species are collected in various areas of the western United States for examination by customary virological techniques.

A preliminary survey for antibodies to Powassan virus has been conducted with sera obtained from the serology departments of the Minnesota, North Dakota, and Wyoming State Health Departments, and a survey for virus has been made of ticks collected in Nebraska, Minnesota, North Dakota, and South Dakota.

Major findings:

1. Western equine encephalitis. Although WEE virus was readily isolated from Culex tarsalis collected in Vale, Oregon, in the midsummers of 1960 and 1961, virus could not be isolated from C. tarsalis collected between October 1960 and May 1961. Since it was also shown that during April and May 1961 mosquitoes readily took blood from chickens, the possibility of a bird-mosquito cycle during this period did exist. It is concluded that there was little evidence of latent infection in birds during the spring months.

2. During the summer of 1960, the Arctic Health Research Center sent tissues from 204 birds and sera from 63 birds. No virus was isolated from tissues, and all sera were free of WEE antibodies. Dr. Salden sent sera from 31 birds from St. Paul Island, but these were free of antibodies against WEE virus. Evidence to date does not indicate that WEE virus reaches Alaska.

3. Since the last report, tests have been completed on tissues obtained from 285 birds at Vale, Oregon, during October 1960, and sera from 173 birds. No virus was isolated from any bird tissue, and only English sparrows and blackbirds had sera with WEE antibodies. The results of examination of bird tissue during 1961 were similar to those obtained in previous years and give no support to the hypothesis of latent infection in birds.

4. During the past 2 summers, there has been little evidence of WEE virus activity in Bismarck, North Dakota. During the summer of 1960, 5,374 C. tarsalis were collected; only 2 isolations of WEE virus were made, and none of 24 sentinel chickens developed WEE antibodies. In Minnesota, adjacent to North Dakota, only 5% of 105 sentinel pigeons developed WEE antibodies during a summer's exposure. During the summer of 1961, only 1 strain of WEE virus was isolated from 2,960 C. tarsalis, and only 1 of 12 sentinel chickens had a low antibody titer against WEE virus at the end of the summer. The low incidence of virus recovered from C. tarsalis in this region and the low incidence of WEE antibodies in chickens cast doubt on the presence of an effective bird-C. tarsalis

cycle during the past 2 summers. There do not appear to have been enough infected C. tarsalis or infected birds to carry virus from one summer to the next, and some other mechanism for overwintering must be postulated for this area.

5. Work has been continued on the possible role of garter snakes as an overwintering mechanism. Garter snakes exposed to the bite of infected mosquitoes during September and October 1960 had virus in their blood when recovered during the spring and early summer of 1961. Infected snakes, however, have not been found in nature.

6. St. Louis encephalitis. Only one isolation of SLE virus was made during 1961; this was at Council, Idaho, and, during 1960, only one isolation was made at Vale, Oregon. The incidence of WEE virus was high in Oregon and Council, Idaho, the past 2 summers, while SLE virus was practically absent. One must assume that there is a marked difference in the summer maintenance of these 2 viruses, the nature of which is presently not clear.

7. Eastern equine encephalitis (EEE). Although recently EEE virus was isolated from turkeys in Wisconsin, and antibodies have been found in birds throughout this state, we were unable to find antibodies in turkeys in North Dakota and pigeons in Minnesota.

8. Other mosquito-borne viruses. No isolations of trivittatus and inornata viruses were made during the summer of 1961. Antibody surveys conducted on sera from cattle, hogs, chickens, and turkeys showed no antibodies against either virus. To date, evidence indicates that inornata virus is not of any importance in causing disease in either man or his domestic animals. Previous studies have shown that approximately 5% of people have trivittatus antibodies.

9. California virus. During studies in the Bitterroot Valley the past season, California virus has been isolated from Haemaphysalis leporis-palustris ticks collected off a snowshoe hare and from D. andersoni collected off a golden-mantled ground squirrel and a chipmunk. Antibodies have been found in 1 of 20 sera from the golden-mantled ground squirrel, 3 of 30 sera from the Columbian ground squirrel, and 6 of 49 from snowshoe hares.

10. Powassan virus. Of 1,058 human sera examined for antibodies to this virus, 2 sera were shown to have neutralization indices of 1,000 or greater. Three sera were found to have neutralization indices of 50 to 100 and one serum a neutralization index of 30. Of those with an index over 1,000, 1 serum was obtained from a person who lived in western Minnesota, the other from a person living in central North Dakota. Those having sera with low indices were Indians who lived on a reservation in Wyoming. One isolation of virus was obtained from 322 D. andersoni collected in the Black Hills of South Dakota during May 1961. In a

survey in the same area later in August, neutralizing antibodies were found in the sera of 4 chipmunks, 3 pools of serum from Microtus and 3 from Peromyscus.

11. A reference collection comprising approximately 60 of the more commonly found arbor viruses has been established, and CF antigens and HI antigens have been prepared as well as mouse immune sera. With these reagents, isolates of the Powassan virus, Anopheles A virus, and Turlock virus have been identified.

Significance:

In contrast to knowledge of the ecology of tick-transmitted viruses, that of mosquito-transmitted virus has not been elucidated completely. With respect to WEE virus, it is now known that every year virus appears in northwestern United States sometime in June or early July, and it is very questionable that either hibernating C. tarsalis or latent infection in birds can account for the appearance each summer. If the problem of overwintering of WEE virus can be solved, it may lead to an understanding of the overwintering of many other mosquito-transmitted viruses and possibly to that of Phlebotomus- and Culicoides-transmitted viruses.

The findings regarding California virus suggest that ticks may play some role in maintenance.

In other parts of the world, viruses of the RSSE group have been of great importance in causing severe human disease. The discovery of a member of this group necessitates thorough study to obtain knowledge of its importance as a cause of human disease in this country and of its ecology. Data obtained so far indicate that human infection is probably infrequent and that small mammals are important in its maintenance.

Proposed course:

With regard to WEE virus, it is intended to concentrate on field work during June and early July when virus appears and to examine every possible vertebrate host and its ectoparasites in an attempt to determine where the virus overwinters.

With regard to SLE virus, study will be concentrated during the early fall months in years when there is an appreciable amount of virus in C. tarsalis during the summer months. The objective will be to determine what vertebrate hosts are important in the maintenance of the virus during the summer months.

An intensive study of small mammals and their ectoparasites in the Black Hills of South Dakota is planned to explore the ecology of Powassan virus.

Future work will explore the role of the snowshoe hare, the cottontail rabbit, and particularly HLP ticks in the maintenance of California virus.

Serial No. NIAID-180

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Olson, T. A., Kennedy, R. C., Rueger, M. E., Price, R. D., and Schlottman, L. L.: Evaluation of activity of viral encephalitides in Minnesota through measurement of pigeon antibody response. Am. J. Trop. Med. and Hyg. 10(2): 266-270. March 1961.

Burgdorfer, W., Newhouse, V. F., and Thomas, L. A.: Isolation of California encephalitis virus from the blood of a snowshoe hare (*Lepus americanus*) in western Montana. Am. J. Hyg. 73(3): 344-349. May 1961.

Honors and Awards relating to this project:

Dr. C. M. Eklund

Appointed to membership in the American Epidemiological Society.

Requested to take part in CDC sponsored course on Principles of Epidemiology in Helena and Billings, Montana.

Serial No. NIAID-181
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Colorado tick fever

Principal Investigator: C. M. Eklund

Other Investigators: W. Burgdorfer, R. C. Kennedy, G. M. Kohls, D. B. Lackman, and L. A. Thomas

Cooperating Units: None

Man Years (calendar year 1961):
Total: 7.0
Professional: 1.0
Other: 6.0

Project Description:

Objectives:

Attempts are being made to determine the incidence of central nervous system involvement and severe hemorrhagic syndromes with infection with the virus of Colorado tick fever (CTF) and also to get some insight into the pathogenesis of these syndromes. Attempts are being made to produce an effective vaccine with suckling mouse brain as the starting material.

Methods:

Any blood specimen from a patient with a history of exposure to ticks is examined for the presence of CTF virus. In this manner, information is obtained regarding clinical syndromes, the annual incidence, and the geographic distribution of the disease.

The pathogenesis of CTF infections is studied by inoculating mice of different ages with small amounts of virus and determining the virus concentrations in various tissues at periodic intervals.

For the preparation of vaccines, suckling mouse brain suspensions are first purified to some extent by treatment with calcium phosphate according to the method of Gordon Smith. They are then inactivated with 1:6000 formalin at 4°C. The potency of the vaccine is checked by

Part B included: Yes

inoculating adult mice twice with 0.5 ml of vaccine at a 1-week interval. Two weeks after the second inoculation, mice are bled to obtain serum for the detection of neutralizing antibodies and then challenged intracerebrally.

Ecologic studies consist of attempts to isolate virus from adult and immature ticks and from their animal hosts.

Major findings:

1. During the past year the incidence of CTF was relatively low, and diagnosis was established by virus isolation in only 23 patients. All had the usual diphasic clinical course. Data were obtained on 2 patients who had thrombocytopenia. This is of great interest since previous evidence obtained by this laboratory indicates that the hemorrhagic syndromes are probably due to thrombocytopenia.

2. Vaccine, prepared as above, has been tested in 27 human volunteers at the Montana State Prison in dosages of either 0.5 ml or 1.0 ml. Two injections were given subcutaneously at a 1-month interval. Twenty-five of the volunteers, one month after the last injection, had sera which neutralized from 2-3 logs of CTF virus in an intracerebral neutralization test in 21-day-old mice. The serum of one volunteer neutralized 50 LD_{50} and the serum of another had no antibodies. None of the volunteers had any ill effects of any kind following the use of vaccine. It has been shown that this vaccine does not produce allergic encephalomyelitis in guinea pigs although brain suspensions from adult mice and guinea pigs do. A vaccine prepared in a similar manner from TP-21, the Malayan strain of RSSE virus, is equally potent in mice.

3. In the Black Hills of South Dakota, chipmunks, Peromyscus, and red squirrels were shown to be of importance in maintaining CTF virus. The important vector there, as elsewhere, is Dermacentor andersoni. Dr. Clifford has isolated CTF virus from Haemaphysalis leporis-palustris ticks collected in Wyoming from a cottontail rabbit. This tick has a wide geographic distribution in North America, and further investigations are desirable on its role in maintenance and transmission of the virus, especially in areas where D. andersoni does not occur.

Significance:

Up to the present time, the only opportunity for American workers to study the clinical picture and ecology of a tick-transmitted virus disease is that created by CTF. The virus has been shown to be maintained by larval and nymphal ticks and their small mammalian hosts. The effect of the virus growth on white blood cells and on the platelets and at times on the red blood cells of human beings is of great interest, as is also the late appearance of antibodies. A marked depressing effect on the bone marrow and on the antibody-forming apparatus is suggested. Such findings might have medical significance in the treatment of Hodgkin's

disease, leukemia, and multiple myeloma.

Proposed course:

The main objectives of the study of CTF have been accomplished, and future work will consist mainly of an attempt to determine the incidence of unusual clinical syndromes. On the basis of work on the course of infection in mice and hamsters of various ages, an attempt will be made to explain the pathogenesis of the disease in experimental animals and man.

Serial No. NIAID-181

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Eklund, C. M., Kennedy, R. C., and Casey, M.: Colorado tick fever. Rocky Mtn. Med. J. 58(6): 21-25 & 44. June 1961.

In press:

Eklund, C. M., Kohls, G. M., Jellison, W. L., Burgdorfer, W., Kennedy, R. C., and Thomas, L. A.: The clinical and ecological aspects of Colorado tick fever. Proc. 6th Intern. Cong. Trop. Med. and Malaria, Lisbon, Sept. 1958.

Eklund, C. M. and Kennedy, R. C.: Preliminary studies of pathogenesis of Colorado tick fever virus infection of mice. Symposium on the Biology of Viruses of the Tick-borne Encephalitis Complex, Smolenice, Czechoslovakia, Oct. 11-14, 1960.

Eklund, C. M., Kohls, G. M., and Kennedy, R. C.: Lack of evidence for transovarial transmission of Colorado tick fever virus in *Dermacentor andersoni*. Proc. Symposium on the Biology of Viruses of the Tick-borne Encephalitis Complex, Smolenice, Czechoslovakia, Oct. 11-14, 1960.

Eklund, C. M.: Mosquito- and tick-transmitted virus diseases and Q fever, a rickettsial disease. Proc. 29th Annual Convention of the American Society of Medical Technologists, Seattle, Wn., June 11-16, 1961, and Am. J. Med. Tech.

Eklund, C. M.: Natural history of Colorado tick fever virus. Journal-Lancet.

Serial No. NIAID-182
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: The epidemiology of human infections of special interest

Principal Investigator: R. N. Philip

Other Investigators: C. M. Eklund and D. B. Lackman

Cooperating Units: Arctic Health Research Center, Anchorage, Alaska;
Tuberculosis Program, USPHS (Dr. Geo. W. Comstock);
Montana State University (Dr. Pat McCarthy)

Man Years (calendar year 1961):
Total: 3.0
Professional: 1.0
Other: 2.0

Project Description:

Objectives:

Studies in this project are directed toward acquiring basic information about the epidemiology and prophylaxis of certain diseases of special interest in which the human being is an important link in the chain of infection.

Methods:

Field investigations coordinated with diagnostic services by the serologic unit are emphasized. The methods used are standard epidemiologic procedures supported by serologic techniques which in 1961 included complement-fixation (CF), hemagglutination, hemagglutination-inhibition (HI), heterophile agglutination, bacterial agglutination, and anti-streptolysin "O" determinations.

Major findings:

1. Historical information was assembled on the occurrence of influenza in Alaska in 1918-19. HI tests on serums obtained from residents of all ages in a number of native villages showed that the prevalence and levels of influenza A/swine antibodies correlated with outbreaks in 1918-19 not only as to their origin in time but also as to

Part B included: No

village of occurrence. These findings are additional evidence that the 1918-19 influenza virus was antigenically related to type A/swine virus.

2. During a study of the etiology of corneal scars present among 40 percent of Eskimo children in southwestern Alaska, serums were tested for adenovirus, herpes simplex and psittacosis CF antibodies. The prevalence or levels of antibodies to these agents among the group of children with scars did not differ from that of a matched control group without scars. Gross and biomicroscopic examination by an ophthalmologist of a selected group of these children suggested the likelihood that most scars originated from phlyctenular kerato conjunctivitis. A close association of corneal scarring with tuberculin skin sensitivity was found which suggests that most cases of this disease are related in some manner to tuberculosis infection.

3. In 1961, specimens from various sources referred for serologic study included 9 cases of Rocky Mountain spotted fever, 10 Q fever, 5 tularemia, 15 Colorado tick fever, one primary atypical pneumonia, and one case of psittacosis.

4. Antistreptolysin "O" titrations of serums from rural residents of the Bitterroot Valley showed levels which were comparable to the high titers found in serums obtained from remote Alaskan native communities.

5. Arrangements were made to obtain serums from a cross-section of the freshman class at Montana State University. These will be held for reference purposes until such time as respiratory or other outbreaks of unusual interest (e.g. influenza) occur. Clinical surveillance of these students will be maintained by the student medical service, and epidemiologic study of outbreaks will be carried out by RML personnel.

Significance:

Although diseases in nature are of primary interest to RML, opportunities occasionally arise for acquiring new information on diseases transmitted from person to person. The rural living and relative isolation of some population groups in the Northwest may affect certain aspects of the epidemiology of some of these diseases. Although this project is somewhat opportunistic in nature and dependent in large measure on existing health agencies in this region for information on occurrence of diseases, the basic research aspects of investigation, rather than surveillance and applied epidemiology, are stressed. The identification of infectious diseases of interest to the field investigation program of the Rocky Mountain Laboratory is the primary reason for maintaining a reference diagnostic laboratory service. However, the service also provides a means for maintaining contact with physicians and public health laboratories in the region.

The finding that in Alaska antibodies to influenza A/swine were limited to persons living at the time of the 1918-19 influenza epidemic

and residing in villages where outbreaks occurred supports the contention that the A/swine strain should be maintained in vaccines in the eventuality that type A virus may antigenically revert to strains resembling the 1918-19 prototype.

Proposed course:

The study of scarring among Alaskan Eskimo children will be completed during the coming year. On assumption that phlyctenular kerato conjunctivitis is etiolologically related to tuberculosis infection, a controlled study of isoniazid prophylaxis against scarring was initiated. Field activities have been completed but the data have yet to be analyzed.

Additional techniques will be utilized in studying the antigens and antibodies of concern to persons responsible for making laboratory diagnoses of infectious diseases. In the immediate future more use will be made of the fluorescent antibody technique and immunoelectrophoresis.

The incidence of "collogen diseases" in the northern Rocky Mountain region has always been a matter of speculation. Although the work here has been confined to antistreptolysin "O" determinations, it is intended that it be extended into the field of nucleic acid serology. This will provide laboratory support for any epidemiological investigations which may be undertaken in the future.

Serial No. NIAID-183
Rocky Mountain Laboratory
Hamilton, Montana

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Comparative Pathology

Principal Investigator: W. J. Hadlow

Other Investigators: None

Cooperating Units: Agricultural Research Council Field Station, Compton, Berkshire, England (Dr. R. L. Chandler) and Epidemiology Branch, NINDB, NIH (Dr. L. T. Kurland)

Man Years (calendar year 1961):
Total: (New project)
Professional:
Other:

Project Description:

Objectives:

To allow for flexibility in its development, this project has the following broad objectives:

(1) To study systematically, with emphasis on the nature of the host response and on pathogenesis, those poorly-understood spontaneous diseases of animals (domestic, laboratory, and wild) that seem analogous to important human diseases.

(2) To investigate experimentally the relation of long-latency viruses to the evolution of protracted degenerative diseases and malignant neoplasms of animals that may have important counterparts in man.

Methods:

Initially, the methods used will be the various techniques usually available in a laboratory of pathology. Certain aspects of the study will require special biochemical and microbiological techniques. Depending on the course of the project, techniques of the biophysicist and the epidemiologist may be found essential.

Part B included: Yes

Major findings:

None. New project.

Significance:

The so-called chronic degenerative disorders, congenital defects, and cancer, not acute infectious diseases, are the main human health problems in the United States today. These broad groups include many specific pathologic entities that are not separable from biological phenomena customarily dealt with in the research activities of the Institute; they merit more attention by persons working in the fields of microbiology and allergy. Indeed, investigation of these killing and crippling diseases might well form a sustaining part of any long-range research program at the Rocky Mountain Laboratory.

One eminently worthwhile, and hitherto largely neglected, approach to these problems is the study of broadly comparable diseases occurring spontaneously in animals. Some closely simulate human conditions not readily duplicated experimentally, whereas others associated with transmissible agents serve as useful laboratory models or prototypes of human diseases. Concern for these naturally-occurring disorders of animals would seem to be a logical, and refreshing, extension of the abiding interest at RML in the interrelation of human and animal diseases. Ostensibly, the broadened concept of disease embraced by comparative pathology has the virtue of being potentially more fruitful in the total effort to elucidate current bio-medical problems than the traditional, and much over-worked, concept of the zoonoses.

Proposed course:

At this time, the exact course of the project is not easily defined; it will depend largely on the availability of adequate facilities for housing large animals (sheep, goats, pigs, and calves) and on the number of investigators in other disciplines who can be interested in contributing to specific phases of the project. Liaison with neighboring veterinary institutions also will strengthen the project.

1. Scrapie, a subacute progressive degenerative disease of the central nervous system of sheep and goats, will be studied first. Caused by an unusual virus having a long period of latency, this disease can be readily induced experimentally, particularly in goats. The natural history and pathologic changes of scrapie bear a striking resemblance to features of several etiologically-obscure neurologic disorders of man. Accordingly, experimentally-induced scrapie will be used as a prototype of these broadly comparable human conditions that may involve similar pathogenetic mechanisms. Conducted in cooperation with Dr. C. M. Eklund, the study will be directed largely toward understanding the pathogenesis of the disease chiefly by the use of accepted techniques of virology.

Much of the work will be done with a mouse-adapted "isolate" of the goat-passage agent. In addition, the pathology of the encephalopathy occurring in mice inoculated with the agent will be studied in cooperation with Dr. R. L. Chandler, Agricultural Research Council Field Station, Compton, Berkshire, England.

2. Other spontaneous diseases of animals that merit consideration in the context of this project include the following:

(a) Lymphosarcoma of cattle. Present evidence strongly suggests that this bovine disease is caused by a virus having a long period of latency.

(b) Aleutian disease of mink, a condition bearing some resemblance to the so-called collagen diseases of man.

(c) Visna of sheep, a virus-induced chronic neurologic disease that pathologically resembles disseminated encephalitis of man.

(d) Arteritis in several species. (A viral arteritis has been observed in the horse.)

(e) Pulmonary adenomatosis of sheep, a chronic progressive disease most likely caused by a virus.

(f) So-called rheumatoid arthritis of swine that may be accompanied by cardiac lesions and vascular changes resembling polyarteritis nodosa.

3. Neuropathologic studies on various animals will be conducted as part of a cooperative venture with Dr. L. T. Kurland, Epidemiology Branch, NINDB, on the possible relation of cycad nut intoxication to serious neurologic diseases (especially Parkinsonism) and associated abnormalities affecting the Chamorro people on Guam.

Serial No. NIAID-183

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

In press:

Hadlow, W. J.: Hereditary diseases of muscle of animals. Symposium on Genetic Neurology (Myopathies).

Honors and Awards relating to this project:

Dr. W. J. Hadlow

Invited to participate in Symposium on Comparative Neuropathology at VII International Congress on Neurology, Rome, and a Symposium on Genetic Neurology at the IV International Congress of Neuropathology, Munich.

Invited to present 3 or 4 seminars to graduate students and staff of the Dept. of Veterinary Pathology, Washington State University, Pullman, sometime in January 1962. Tentative topics: Pathology of Scrapie; Pathology of Salmon Poisoning; Survey of Diseases of Muscle of Animals.

LABORATORY OF BACTERIAL DISEASES

Summary 1

200 - Brucellosis 3
201 - Basic Studies on Staphylococcus 7
202 - Identification of Pseudomonas 9
204 - Intracellular Parasitism 12
205 - The Role of Infection in the Delayed Deaths of Mice Following Extensive Burn Injury 14
206 - Bacterial Food Poisoning 17

SUMMARY OF ACTIVITIES
Laboratory of Bacterial Diseases
December 1961

The research program in the Laboratory of Bacterial Diseases has continued to develop in the same general areas noted in last year's report. The major emphasis has been on the projects on Intracellular Parasitism and Staphylococcus.

In the course of the project on Intracellular Parasitism a study was undertaken of the production of specific antibody by mammalian cells in vitro. This study has been reasonably rewarding and offers considerable promise. Accordingly, at present, the major effort on this project is devoted to this line of investigation.

Specifically, cells are obtained from the peritoneal cavity of laboratory animals, usually the guinea pig. A method has been developed which yields a high proportion of mononuclear cells or macrophages. Further separation of the cell types results in explants which are composed almost entirely of this type of cell. Cells removed from immune animals and properly handled and cultured elaborate specific antibody in vitro for days or even several weeks. The explants result in growth of sheets of cells which are derived from the mononuclear cells or macrophages. On glass they become stellate cells but retain some of the characteristics of macrophages. Such tissue cultures can be maintained for long periods and be carried through at least a number of subcultures. The antigen usually has been egg albumen; the test procedure hemagglutination of specifically sensitized red blood cells. While production of antibody in vitro has been sporadic, significant titers have been obtained, and in a number of experiments antibody has been detected for periods of several weeks. In comparison with initial cell homogenates and with supernates from cells maintained under non-optimum conditions, the amount of antibody produced exceeds that which can be accounted for by release of preformed antibody and certainly represents new protein synthesis after removal of the cells from the animal. There is still a question as to the type of cell which is producing antibody and also whether these particular cells have undergone multiplication even though general growth of the tissue culture has occurred. This system allows of further study of the factors involved in continued antibody production.

In the project on Staphylococcus there is continued emphasis on the factors responsible for virulence of the organism and on basic studies of methods of identification of pathogenic strains of the organism. Gel-diffusion immune precipitation techniques have been applied to standard reference strains of Staphylococcus. Employing methods which tend to eliminate minor and non-specific cross reactions the antigenic mosaic of such strains has been characterized. This technique seems promising for the development of a system of identification. If reference spectra consisting of precipitation patterns formed with specific reference antigens and antisera can be established for staphylococci, this would form a solid basis for the identification of strains.

We continue to collaborate with other Brucella research centers throughout the world in the development and standardization of laboratory tests for species identification and for diagnosis of Brucella infection. Brucellosis is still a disease of world wide importance in relation to the welfare of mankind. Control of the disease in domestic animals rests in part upon regional epidemiological studies which require precise identification and classification of strains. A unified effort is currently under way in an attempt to clarify Brucella taxonomy. This undertaking is under the aegis of the Subcommittee on Taxonomy of the Genus Brucella of the International Committee on Bacteriological Nomenclature, of which the Chief, Laboratory of Bacterial Diseases is a member.

A paper on the antigenic structure and typing of Pseudomonas has been published during the year. This paper presents an antigenic schema for the serological identification of Pseudomonas strains more commonly found in human infection. Its appearance has generated considerable enthusiasm and numerous requests for reprints. Two further detailed papers in this area are in preparation.

There remains a continuing need for further monetary support, space, and personnel to pursue the basic studies mentioned. Further, there are other areas within the province of this laboratory which should be developed were space, personnel, and monetary support available. Among these are the diarrheal diseases, bacterial genetics, germ-free research, and the whole field of the PPLO and the L forms of bacteria.

Serial No. NIAID-200
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Brucellosis

Principal Investigator: Norman B. McCullough, M. D.

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 1-1/2
Professional: 1/2
Other: 1

Project Description:

Objectives:

The objectives of this project are broad and studies are conducted on all aspects of brucellosis, including the nutrition, metabolism, and enzymatic make-up of the organism, mechanism of action of antibiotics on the organism, factors determining virulence of the organism, the development and efficacy of prophylactic immunizing agents, field studies to determine the occupational hazard of the disease, epidemiology, diagnosis and therapy of the disease in man.

Clinical studies concern diagnostic criteria for the disease in man, especially in borderline cases of chronic illness in which present cultural and serological techniques fail to provide reliable evidence of infection; studies of the pathogenesis of the disease; and the evaluation of current therapeutic regimens.

Part B included.

Methods Employed:

The Warburg technique is employed in enzymatic and metabolic studies; growth in a synthetic medium is used for nutrition experiments; guinea pig inoculation for virulence and vaccine studies; occupationally exposed groups for epidemiological studies; human patients for studies on diagnosis and therapy.

Major Findings:

Due to the press of other studies, and limitation of personnel, the effort expended on this project has been markedly reduced. Work on the taxonomic problems presented by Brucella has continued. These results will contribute to the considerations of the Subcommittee on Taxonomy of Brucella of the International Committee on Bacterial Nomenclature which meets next summer and of which the senior investigator is a member.

Some studies have been done on the viability and antigenicity of Russian Brucella Vaccine for human use. These results will be reported to and presumably will be considered by the World Health Organization in determining the utility of this vaccine.

Significance to the Program of the Institute:

In terms of world-wide significance, brucellosis ranks in incidence and importance with malaria, tuberculosis, and parasitic diseases, in relation to the welfare of mankind. Even in the United States, by reported incidence, it is the most important disease of animals transmissible to man, is the cause of serious economic loss to the livestock industry, is an occupational disease, and one which presents many problems in the diagnosis and therapy of the infection in man. The organism readily lends itself to basic studies outlined elsewhere in this report.

Proposed Course of Project:

Continue studies under way.

Continue to collaborate with other Brucella research centers throughout the world in the development and standardization of laboratory tests for species identification, and for diagnosis of Brucella infection.

Study of Brucella bacteriophage as regards basic phenomena of bacteriophagy and potential use of phage for preparation of enzymatic and subcellular fractions of Brucella for biochemical studies, and as possible immunizing agents. Investigate the utility of specific phages as an aid in identification of Brucella species and strains.

Continue to use Brucella as a basic tool in the study of mechanism of development of acquired resistance to antibiotics and in studies of intracellular parasitism.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Tobie, J. E.; and McCullough, N. B.: Serologic Evidence of *Leptospira pomona* Infections in Meat Inspectors. Jour. Am. Vet. Med. Assn. 138: 434-436, 1961.

Serial No. NIAID-201
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Basic Studies on Staphylococcus

Principal Investigator: Elizabeth Verder, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 9/12
Professional: 3/12
Other: 6/12

Project Description:

Objectives:

1. To develop an objective means of measuring virulence of Staphylococci.

2. To determine the factors responsible for virulence.

3. To develop more satisfactory methods for the identification of individual strains used in measuring virulence.

Methods Employed:

Use of various physical and chemical methods in separation of antigenic components of cells or their products to permit assessment as factors in pathogenicity or toxicity.

Part B not included.

Application of various serological methods, previously described by investigators including gel diffusion techniques as well as agglutination, hemagglutination and precipitin tests, to the study of these fractions with the hope of detecting antigenic components for use in the identification of specific strains.

Major Findings:

The preliminary work on the precipitinogenic relationships between various strains of staphylococci appears to offer promise as one means of differentiation among strains.

Significance to the Program of the Institute:

The characterization of virulent strains of staphylococci and the identification of individual strains continue to be important problems. A factor of prime importance to the invasive capacity of a strain in necrotic tissue may be an unimportant characteristic of a virulent strain in another infectious process.

Proposed Course of Project:

As described above.

Serial No. NIAID-202
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Identification of Pseudomonas

Principal Investigator: Elizabeth Verder, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 7/12
Professional: 3/12
Other: 4/12

Project Description:

Objectives:

a) Identification of strains for other scientists.

b) Orientation of scientists in methods used for study of biochemical characteristics of various species and for serological typing.

c) Distribution of cultures of strains of various species and of representative serological types of *Ps. aeruginosa* on request to research scientists.

Part B included.

Methods Employed:

The methods which we have employed are ones developed or modified in the course of our studies on the classification of *Pseudomonas*; the studies were initiated during work on our project on diarrheal disease of the new born and have been carried on for eight years. A proposed scheme for the antigenic analysis of strains of *Ps. aeruginosa* was worked out and has been employed successfully in identifying strains by serological typing.

Major Findings:

Methods have been established for use in the identification of individual strains of *Pseudomonas* so that epidemiological surveys in relation to the spread of resistant strains may be carried out successfully.

Significance to the Program of the Institute:

Methods have been developed for studies on the spread of this group of antibiotic resistant organisms. These bacteria are of considerable medical importance, particularly in infections in individuals with minimal resistance such as newborns, the aged, children with leukemia, individuals with extensive or deep burns, etc. With the increasing importance of Pseudomonas infections the findings in these studies are receiving recognition in clinical applications.

These studies are of importance in connection with the establishment of colonies of animals free of organisms of this genus. Animals carrying some strains cannot be used for irradiation studies because of high mortality from induced septicemias.

Requests for additional information regarding our studies have increased since the publication of our paper on the proposed serologic schema for *Ps. aeruginosa*. Requests for cultures have also increased.

Proposed Course of Project:

Two additional papers are being prepared for publication.

Serial No. NIAID-202

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Verder, A. E.; and Evans, J. E.: A Proposed Antigenic Schema for the Identification of Strains of Pseudomonas aeruginosa. Jour. Inf. Dis. 109: 183-193, 1961.

Serial No. NIAID-204
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Intracellular Parasitism

Principal Investigator: Norman B. McCullough, M. D.

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1961):
Total: 2-1/2
Professional: 1/2
Other: 2

Project Description:

Objectives:

To study host-parasite relationships in intracellular metabolism for the purpose of increasing our understanding of disease and immunity.

1. To determine the effect of infecting organisms on the metabolism of the infected cell.

2. To determine the effect of certain bacterial products, such as Staphylococcus toxin and bacterial fractions, on cells maintained as explants and in tissue culture, and upon certain subcellular particles.

3. To determine the contribution of cells, and of subcellular particles, extracts, and enzyme systems to the nutrition of and their effects on the characteristics of intracellular bacteria.

Part B not included.

4. To study protein synthesis (such as antibody production) by cells from normal and immunized animals.

Methods Employed:

The study employes various pathogenic agents such as Brucella, Staphylococcus, *Pasteurella tularensis*, and certain viruses. It entails the use of standard tissue culture techniques, chemically defined media for bacteria, the Warburg technique, standard chemical procedures, phase microscopy, fluorescent antibody technique, vital dyes, electrophoresis, and chromatography.

Major Findings:

During the last year the major effort has been a study of antibody production *in vitro* by cells, presumably macrophages, derived from the peritoneal cavity of the guinea pig. Using hemagglutination of sensitized tanned red cells as indicator, cells taken from guinea pigs immunized with egg albumen release antibody *in vitro*. While results have been somewhat erratic, in a number of experiments, antibody production has continued for days or even several weeks, periods in excess of those found with control tubes in which the cells remained alive but were not allowed to grow. Such antibody release has been in excess of the amount present in the cells when harvested, and hence represents new synthesis of antibody by the cells *in vitro*. Production of antibody appears to be correlated with growth of the cells and continues beyond the time when the cells, on glass, have assumed a stellate form. This morphological change in the cell form does not in itself appear to be associated with cessation of antibody production. There has been great improvement in the technique of harvesting and preparing the cell cultures, and antibody production is being demonstrated more consistently and for longer periods. We now have a system which appears reproducible enough to allow of definitive study of the many factors involved in the successful production of antibody by cells *in vitro*.

Significance to the Program of the Institute:

This study concerns the basic interactions between host cell and infecting agent and hence any contribution to knowledge would be of significance to the entire field of infectious diseases and to the program of the Institute.

Proposed Course of Project:

As stated above. Continued emphasis will be placed on the study of antibody production *in vitro*.

Serial No. NIAID-205
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: The Role of Infection in the Delayed Deaths of Mice Following Extensive Burn Injury

Principal Investigator: Elizabeth Verder, Ph.D.

Other Investigator: None

Cooperating Units: Drs. Sanford Rosenthal and Carl Millican

Man Years (calendar year 1961):
Total: 3/12
Professional: 3/12
Other: 0

Project Description:

Objectives:

a) To review literature dealing with increased susceptibility to infection following traumatic shock, radiation and extensive thermal injury.

b) To evaluate findings in extensive studies, carried out in 1943, on incidence of bacteria in tissues of burned mice and their role in infectious processes causing delayed deaths in mice following extensive burn injury.

Methods Employed:

Standardized techniques for the isolation and identification of bacteria from infectious processes were employed.

Part B included.

Major Findings:

Studies indicate that generalized infection is an important factor in the delayed deaths in mice during the three weeks following extensive thermal injury. Bacteria of low virulence and invasiveness apparently spread both from previously localized foci of latent infection and from areas of colonization on damaged surfaces.

Significance to the Program of the Institute:

Septicemias and other fatal infections are of increasing importance as the cause of death following extensive burn injury in spite of advances in treatment.

Proposed Course of Project:

Terminated.

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Verder, A. E.; and Rosenthal, S. M.: The Role of Infection in the Delayed Deaths of Mice Following Extensive Burn Injury. Proc. Soc. Exp. Biol. & Med. In Press.

Serial No. NIAID-206
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1961

Part A.

Project Title: Bacterial Food Poisoning

Principal Investigator: Elizabeth Verder, Ph.D.

Other Investigators: None

Cooperating Units: Varies with each outbreak. May be epidemiologists, sanitary engineers, clinicians, etc.

Man Years (calendar year 1961):
Total: 5/12
Professional: 3/12
Other: 2/12

Project Description:

Objectives:

a) To isolate and study all bacteria with properties of potential causative agents from 1) persons developing symptoms of food poisoning, 2) foods that could have served as vehicles of agent or agents and 3) the food-handlers involved.

b) To correlate laboratory data with findings of other investigators studying the outbreak.

Methods Employed:

Standardized techniques for the isolation and study of bacteria which may or may not have been associated with food-poisoning previously but which have some of the characteristics of potential causative agents.

Part B included.

Major Findings:

a) Evaluation of data collected in bacteriological and serological studies of organisms of the Arizona group associated with a food-borne outbreak of gastroenteritis.

b) Inconclusive studies on an outbreak in which nine in a group of eighty PHS sanitary engineers became ill.

Significance to the Program of the Institute:

With a large increase in the number of cooked or partially cooked foods which now are available in a chilled or frozen state, and with the popularity of both hot and cold "carry-out" food, food-poisoning due either to infectious agents or to their toxins may become a more important public health problem.

Proposed Course of Project:

As outlined.

Serial No. NIAID-206

PHS-NIH
Individual Project Report
Calendar Year 1961

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Verder, A. E.: Bacteriological and Serological Studies of Organisms of the Arizona Group Associated with a Food-Borne Outbreak of Gastroenteritis. Journal of Food Science. In Press.

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